SPONTANEOUS AND EXPERIMENTAL

LEUKÆMIA IN ANIMALS

LADY TATA MEMORIAL TRUST

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BY

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OLIVER AND BOYD

EDINBURGH TWEEDDALE COURT

LONDON 98 GREAT RUSSELL STREET W C

1942

PRINTED IN GREAT BRITAIN BY
OLIVER AND BOYD LTD EDINBURGH

TRANSLATED FROM DANISH
INTO ENGLISH BY
C L HEEL

PREFACE

This book was written at the invitation of the Scientific Advisory Committee of the Lady Tata Memorial Trust, and is published under the Trust's authority

The Trust was founded in 1932 by the late Sir Dorabji Jamsetji Tata of Bombay, in memory of his wife, the late Lady Tata C B E After payment of the administrative expenses one fifth of the income is devoted to the encouragement—by scholarships or prizes—of scientific research by Indians on any problem which bears directly or indirectly upon the relief of human suffering the remainder is allocated, under normal conditions, in the form of grants or scholarships to workers of any nationality and in any country of the world for research on diseases of the blood with special reference to leukæmia In making the special awards to Indians the Trustees are advised by an Indian Committee under the chairmanship of Dr R Row O B E, in Bombay, and in allocating the funds for international research on blood diseases they have the assistance of the Scientific Advisory Committee whose headquarters are in London the membership of the latter Committee is given on p ii

Since the inception of the Trust's work in 1932, a great volume of important research on blood diseases has been supported in many different countries Among workers thus assisted whose studies have related directly to the problems of animal leukæmia mentioned in this book have been Dr Engelbreth Holm, Dr L Doljanski, Dr Kaalund Jorgensen and Dr Jorgen Bichel in Denmark, Professor Ch Oberling and Dr M Guerin in France, Dr W Bungeler in Germany Dr P A

Gorer, Dr M C G Israels and Professor J McIntosh in Great Britain, Professor K Jarmai in Hungary, Professor E Storti in Italy, and Professor Eugene L Opie and Dr Jacob Furth in the U S A The following pages give plentiful evidence of the outstanding contributions made by research workers thus assisted by the Trust to the development, by experimental methods of our present knowledge of the disease

The problem of leukæmia—difficult if not impossible to investigate experimentally in man—is perhaps even more susceptible than the related problem of cancer to observational and experimental research in animals In addition to the fowl leukæmias, with their suggestive analogy not only with the malignant tumours of fowls but also with other virus diseases, there exists for study a wide range of leukæmic and neoplastic conditions in mammals notably mice which, as the author points out, provide a truly remarkable parallel to corresponding conditions in man, in their diversity of blood pictures and their subtle gradations between the 'pure leukæmic and the 'pure sarcomatous' states The study of these animal diseases from the clinical pathological, biochemical and genetic points of view proceeded with increasing intensity up to the outbreak of the present war and has produced an immense and widely scattered literature Moreover the very fact that the experiments and discoveries relating to a particular aspect of leukæmia may themselves be of absorbing interest to the specialist has perhaps led to some risk of research on the problem of leukæmia in animals being viewed as a series of isolated incidents rather than as a co ordinated whole It was to obviate such danger that the Committee, with the concurrence of the Trustees, invited Dr Engelbreth Holm to undertake the writing of this book The book presents a summary of the collected

knowledge in a readily assimilable and co ordinated form, it is aimed especially at showing the relationship of the different forms of animal leukæmia not only to each other but also to human leukæmia and thus it illustrates the significance of these experimental studies for the ultimate problem of the prevention and treatment of the human disease Apart from their significance for human medicine, these studies of animal leukæmia have an even more direct bearing on problems of animal husbandry

A tribute must be paid here to Dr Claude E Forkner's admirable and comprehensive monograph, *Leukemia and Allied Disorders* (Macmillan Co, New York 1938) in which naturally a good deal of the same ground is covered Dr Forkner's approach to the problem however, is necessarily different, since he is concerned mainly with the human disease, and the present author chiefly with leukæmia in animals, and more particularly with the experimental aspects of the subject

Dr Engelbreth Holm prepared his manuscript in Danish and entrusted the task of its translation into English to Mr C L Heel, formerly of the British Consulate staff in Copenhagen The Committee are grateful to Mr Heel for the skill of his translation, which has made their editorial task comparatively easy The Danish manuscript was completed early in 1940, but with the author's approval certain further references to important publications have been added by the Committee to the English version so as to bring the information as nearly as possible up to date In the work of editing the English manuscript and of making these additions the Committee have had expert help from Professor T Dalling Dr P A Gorer and Dr P R Peacock to whom their thanks are due Grateful acknowledgements are also made to Miss

E Wigmore, B Sc, Librarian of the National Institute for Medical Research, London, who kindly undertook the task of checking the many references

It may be suggested that the present time is unpropitious for researches aimed at the saving of life by the conquest of a disease which is relatively rare in man It is difficult for workers in this country—and doubtless also for those on the continent of Europe—to carry on such investigations effectively in a war time environment, and even in the United States of America the lack of a free international exchange of information and research material, together with the not far distant echoes and rumours of wars, must impose some check on progress This very circumstance, however, may have made the year 1940 especially appropriate for taking stock of the existing knowledge of the experimental leukæmias, since it marks the end of a period of intensive research activity in this field It is to be hoped, when the present war is over, and it becomes possible for research workers throughout the world to resume their attack on the common enemies of mankind —disease and untimely death—that this book will be found to provide a valuable foundation of collective knowledge for the better planning of further researches on leukæmia and in neighbouring fields of investigation dealing with such diseases as cancer It is to such researches that we may hopefully look for life saving discoveries in the future

SCIENTIFIC ADVISORY COMMITTEE
LADY TATA MEMORIAL TRUST
LONDON

December 1941

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INTRODUCTION

HISTORICAL SURVEY

Human leukæmias were recognised as an independent group of diseases in 1845 through Craigie's, Bennett's, and, in particular, Virchow's studies Virchow noted that the pathological picture differed from others in which an increase of white corpuscles is found in the blood and he introduced the name 'Leukämie' By this he understood a diseased condition 'welche sich durch eine progressive und bis zu ganz ungewöhnlichem Masse anwachsende Vermehrung der farblosen Körperchen im Blute charakterisiert' Later on the basis of the changes observed in the organs, he divided leukæmia into two forms, splenic and lymphatic

During the following years there were many reports of the new disease, but it was a long time before its nature, the interrelation of its various forms, and their differentiation from other diseases, were even partly explained

In 1865 Cohnheim reported a case without changes in the blood and put forward the concept 'Pseudo leukämie In 1870 Neumann reported a case with pronounced changes in the bone marrow, thus adding myelogenous leukæmia to Virchow's splenic and lymphatic forms

No more rational classification, however, appeared until the publication in 1879 80 of Ehrlich's studies (summarised in Ehrlich, 1891) had made possible an exact differentiation of the various blood corpuscles and their precursors, in consequence of which leukæmias

could be distinguished far more surely than through the evidence provided by macroscopic examination Research showed, moreover, that there were two main forms—the myelogenous and the lymphatic

Long before this point had been reached it was clear that cases of disease which must be regarded as leukæmia occurred in lower animals as well as in man In 1858 Leisering published a case of leukæmia in a horse, and later other cases were reported, in 1865 he published a case in a pig Bollinger (1871) described leukæmia in pigs and (1874) in a dog, Siedamgrotzky (1871) observed it in a dog and in a cat The first case in a mouse was observed in 1874 by Eberth, and cases in many different kinds of animals have since been reported The disease was first observed in birds (fowls) by Roloff in 1868 and by Caparini in 1896

It was not long before leukæmia became the subject of experimental research, which proceeded side by side with its clinical and pathological investigation In 1872 Mosler attempted the first transmission experiment, by injecting the blood of leukæmic patients into dogs and rabbits In 1874 Bollinger tried to transmit the disease from one dog to another Many transmission experiments were performed, but they were all ineffective until Ellermann and Bang (1908) transmitted leukæmia in fowls from diseased to healthy birds, thereby introducing a new and very important phase in the experimental study of leukæmia

While the studies made before this period are mainly of historical interest, Ellermann and Bang's work was followed by a large number of investigations which attempted to unravel the details of fowl leukæmia, these have included researches into its morbid anatomy and hæmatology and, more particularly, into the nature and properties of the ultramicroscopic, filterable agent which was shown by Ellermann and Bang to be capable

of transmitting the disease to healthy birds without the co-operation of intact cells.

To these studies must be added the very large number of investigations carried out all over the world during the last twenty or thirty years on the subject of leukæmia in mammals, especially in mice and to a lesser extent in guinea pigs, rats and cattle. Most of the branches of medical science have been involved in these investigations—pathology, histology, physiology, biochemistry, genetics, radiology—but the origin of the morbid process in leukæmia has not yet been explained. These studies, however, which have been pursued with increasing intensity in many different centres, have provided an almost overwhelming mass of observations—clinical, pathological, and experimental—which cannot yet be co-ordinated into a comprehensive whole but which, nevertheless, shed light on many sides of the leukæmia problem.

It is the aim, then, of the present work to review the collective knowledge of spontaneous and experimental leukæmias in animals and to discuss the relationship which these conditions bear to the problem of leukæmia in man.

CLASSIFICATION AND NOMENCLATURE

The same problems are involved in the appropriate classification of animal leukæmias as in the case of those occurring in man. Until more is known of the ætiology and genesis of these diseases, the clearest classification will be that based on the type of cell which has undergone proliferation, to produce characteristic changes in the organs and possibly in the blood. Here, difficulties are immediately encountered, because, since the nature of the relationship between the different types of blood cells is still a matter for discussion, a classification of leukæmias on this basis may to some extent depend

on whether one is an adherent of the 'monophyletist', the 'dualist' or the 'polyphyletist', school of thought

For the present it will probably be best to adopt the following terms 1 *stem cell leukæmia*, 2 *erythro leukæmia* (especially in birds) 3 *myelogenous leukæmia*, 4 *lymphogenous leukæmia*, 5 *monocytic leukæmia* To these must be added 6 *plasma cell leukæmia*, occurring in human beings but not observed in animals (Table I)

TABLE I

Classification of Leukæmias

Arising from cells in	Type of Leukæmia	Corresponding Form of Tumour	Dominating Cell form
—	Stem cell leukæmia	—	Undifferentiated stem cell
Bone marrow (*myeloid*) system	Erythroleukæmia	Erythroblastoma	Basophil erythro blast (Ellermann s erythrogonies) Erythroblast
	Myelogenous leukæmia	Chloroma	Myeloblast Myelocyte
	Plasma cell leukæmia (not known in animals)	Plasmacytoma Multiple *myeloma*	Plasma cell pre sumably *from the reticulum of the* marrow
Reticulo endothelial system	Monocytic leukæmia	Reticulo sarcoma	Monoblast (According to the dualists this form is myelogenous)
Lymphatic system	Lymphogenous leukæmia	Lymphosarcoma	Lymphoblast Atypical lymphocyte

In its typical appearance each of the forms named above will display extensive diffuse systematic prolifera tions in the organs The cases are *leukæmic* or *sub leukæmic* (aleukæmic) according as to whether there is

or is not also an increase in the blood of the kind of cell concerned This classification is, however purely clinical, as is the division into acute and chronic cases in accordance with the duration of the disease It has not been possible to demonstrate any essential difference between leukæmic and subleukæmic leukæmia nor between acute and chronic cases, beyond the differences indicated by the names themselves Furthermore, since all possible intermediate forms, as well as cases that change from subleukæmic to leukæmic or from chronic to acute are not infrequently observed these terms however useful from a clinical point of view, are obviously of no importance where the classification of leukæmias is concerned

The forms of which the classification has been—and still is—the subject of most discussion are plasma cell leukæmia, which, as has been mentioned is not known in animals, and monocytic leukæmia

Plasma cell leukæmia may be classified as a special form of myelogenous or lymphogenous leukæmia according to whether the plasma cell is regarded as derived from the myeloblast, the erythroblast or the lymphocyte But, since recent research indicates that the plasma cells in plasma cell leukæmia probably originate in the reticular cells of the bone marrow it seems justifiable to place plasma cell leukæmia in a special group

Similar arguments apply to monocytic leukæmia the dualist school (Naegeli) asserting that the monocyte is derived from the myeloblast, and that these cases therefore rightly belong to the group of myelogenous leukæmias The more general opinion is that the monocyte is an independent type of cell arising, presumably from the reticulo endothelial system For the time being it would seem to be expedient to maintain the independent group 'monocytic leukæmia' (see,

for example, Forkner, 1938), even though the features of this group can hardly be said to be clearly defined

Forkner (1938) proposed a more detailed classification, by subdividing the myelogenous leukæmias into neutrophilocytic, eosinophilocytic, basophilocytic, megakaryocytic leukæmias, chloro leukæmia and erythroleukæmia Such a sharply distinguished classification, however, is inapplicable to the animal leukæmias, if only because leucocytes with neutrophil granules, from which the name of the most important group in Forkner's classification is derived, are not found at all in the lower animals they are replaced in rabbits and fowls, for example, by 'pseudo eosinophil leucocytes' A terminology founded on the special granulations of animal cells may thus create confusion

Besides the typical cases of leukæmia with changes spread diffusely throughout the whole of the myeloid or lymphatic system, pathological pictures will be found in lower animals as in human beings, which are characterised by tumour formations composed of the same cells as are found in excess in leukæmic blood Such tumour formations may be single or multiple Furthermore, transitional forms between these cases and pure leukæmias are not infrequently observed they may be regarded equally well as more or less localised tumour formations with leukæmic blood changes or as cases of leukæmia accompanied by one or more circumscribed tumours

The tumour formations in the myelogenous, lymphogenous and monocytic leukæmic groups are of particular interest The tumours corresponding to myelogenous leukæmia are chloromata which are known especially in pigs Lymphosarcomata (analogous to Kundrat's lymphosarcomatosis without blood changes and to Sternberg's leucosarcomatosis with blood changes,

in human beings) correspond to lymphogenous leukæmia and occur in most animals, they have been investigated in mice and cattle especially Similarly, monocytic leukæmia of man corresponds to the reticulum cell sarcomata and reticulo endotheliomata observed in mice Finally, it should be mentioned that cases with erythroblastomata can be similarly paralleled by erythroleukæmia in fowls

Since Virchow's designation of the group of diseases as 'leukæmia,' a somewhat confused nomenclature has gradually arisen as the different forms have become known Up to the present no uniform logical terminology has been reached Various suggestions have been made for improvements in this respect Many terminologies have been used to a greater or less extent, but only a few have contributed towards the attainment of greater clarity Türk proposed Lymphomatosis' and 'Myelosis' Ellermann and Bang suggested replacing 'Leukæmia with 'Leukosis, a term used especially in German and Scandinavian literature, and more particularly for leukæmia in fowls These proposals were based on the fact that the word 'Leukæmia' indicates a symptom that may very possibly be lacking

Virchow's term leukæmia ('Weisses Blut') is not always accurately descriptive if interpreted literally, but, so long as we have no precise knowledge of the nature of the disease and cannot designate it in accordance with its characteristics, the name leukæmia is quite a practicable one, especially since it has lost its true meaning in the course of time and has become the customary term for all the various forms in which the disease may occur As Forkner points out, the word leukæmia has attained a particular significance just as have other medical terms, *e g* anæmia, which, translated literally are equally incorrect, there is thus no

reason to replace the word leukæmia with the word 'leucocythæmia'

Cohnheim (1865) introduced the word 'pseudo-leukæmia' as a term for subleukæmic cases. This, however, was abandoned after it had been used and misused in various ways. The word was unfortunate, for 'pseudoleukæmia' indicates a condition which resembles leukæmia but is different from it; for this reason Orth tried to replace it with 'aleukæmia', which was soon modified to 'aleukæmic leukæmia' as a name for the pathological picture in which there are no blood changes. It has now appeared that pure 'aleukæmic' cases are extremely rare; also that the total leucocyte count in leukæmia may quite often be normal, although the blood contains the typical abnormal cells. For this reason Forkner (1938) may be right in his proposal to substitute the term 'subleukæmic' for aleukæmic, even though the distinction is not essential.

If a leukæmic pathological picture is to be classified clearly it must be given a name which corresponds to the character of the pathological changes, or possibly to the cell form that distinguishes the picture. Virchow's (1847) division of leukæmia into splenic and lymphatic had to be abandoned because subsequent microscopical investigations showed that it was illogical and impracticable. Neumann (1870) introduced the term 'myelogenous' leukæmia (which included most of Virchow's splenic leukæmias) and the two words myelogenous and *lymphatic have persisted as descriptive titles of the most* important and most frequent forms of leukæmia. Nevertheless, many alterations have since been proposed, such as 'myeloic', 'myeloid' and 'myelocytic' on the one hand, and 'lymphoid' or 'lymphogenous' on the other. The expressions in most general use are 'myelogenous' and 'lymphatic', but Forkner is right when he suggests that it would be logical to replace 'lymphatic',

which corresponds to 'myeloid', with 'lymphogenous' which corresponds to 'myelogenous'. These terms will therefore be used in the present work.

As the study of leukæmia progressed, it gradually became clear that some of the cases described as leukæmia, in animals as well as in man, did not really belong to this group of diseases but were of quite different pathology—*e.g.* septic and certain neoplastic conditions. The blood picture found in such cases was commonly reminiscent of that in leukæmic leukæmias, but the characteristic visceral changes were entirely absent, or changes were observed which resembled those of leukæmia but could, by careful examination, be distinguished from them. Conditions such as these are generally called 'leukæmoid'. The changes with which they are associated may resemble those of leukæmia to a greater or lesser extent but are fundamentally different in nature.

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PART I

SPONTANEOUS LEUKÆMIA IN ANIMALS

CHAPTER I

SPONTANEOUS LEUKÆMIA IN BIRDS

WHILE leukæmia has not been observed in the lower vertebrates, the condition is known in many kinds of birds

Cases of lymphogenous leukæmia have been described in turkeys (Reinhardt, 1925, Cohrs, 1927, Becker, 1928, Jarmai, 1929), geese ducks pigeons (Lund, 1926, Haupt 1928) swans (Geurden, 1934), storks and parrots (Fox 1923), canaries (Satterlee 1906 Haupt, 1928), and vultures (Payer, 1935) It is possible that these cases include some that should more correctly be considered as cases of erythroleukæmia as observed in fowls, for this form is still known by some as lymphoidocytic leukæmia' or 'intravascular lymphoid leucosis', Ellermann's first terms for erythroleukæmia

Cases of erythroleukæmia ('erythroblastosis') which have been thoroughly investigated have been observed in canaries (Farkas 1930, Kogler, 1933) as well as in fowls, and also in parrakeets (Jarmai 1939), in the latter the erythroleukæmic changes in the blood and organs were found to be associated with a fibrosarcoma, as not infrequently occurs in fowls

These cases permit the supposition that leukæmia may be found in most kinds of birds, but no more detailed investigation of its specific incidence has been made Apart from Jarmai's cases in parrakeets, leukæmias in the species mentioned have not been investigated by transmission or any other sort of experimental research



Fig. 1. Liver and spleen from case of spontaneous lymphogenous fowl leukemia (Cf. Fig. 11)



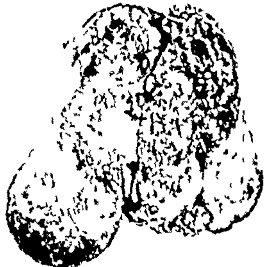


Fig. [illegible]. Liver and spleen from case of spontaneous lymphogenous fowl leukemia (Cf. Fig. 11)

Our knowledge of bird leukæmias is dominated preponderantly by those of domestic fowls, which have been studied by many workers and used for innumerable experiments

Fowl leukæmias have been known very much longer than leukæmias in other sorts of birds The first reported case, one of 'lymphosarcomatosis,' was published by Roloff in 1868 Caparini (1896) described the histological changes in three enlarged fowl livers in the pathological museum of the Veterinary Institute in Naples, which he diagnosed as leukæmic One year later, Moore (1897) described an 'infectious leukemia in fowls, which, however, has no connection whatever with true leukemia, in these cases the blood changes are caused by infection with *Bacterium sanguinarium*, which is also pathogenic for other animals, the severe leucocytosis accompanying the infection has been misinterpreted as leukæmia Moore's report is the first concerning a case of animal disease in which it can be definitely recognised that a 'leukæmoid' blood picture was mistaken for evidence of the leukæmic process Many analogous cases involving different kinds of animals have since been published, in which non specific leucocytosis or lymphocytosis produced by the most widely differing causes has been misinterpreted and reported as leukæmia

Many cases of fowl leukæmia were reported during the following ten years (Butterfield, 1905, Koch and Rabinowitsch, 1907 Kon, 1907, Warthin, 1907, and others), but the disease was not subjected to more detailed investigation

FOWL LEUKÆMIA

Research into fowl leukæmia was directed along an entirely new path in 1908 09 by Ellermann and Bang's epoch making discovery that the disease can be trans

mitted to healthy fowls by intravenous or intraperitoneal inoculation with blood or emulsions of organs from diseased birds, and also by cell free filtrates

This observation, which was made two years before Rous described the transmissibility of fowl sarcoma by means of cells or by a cell free virus, actually represents, in the light of our present knowledge, the original discovery of an avian mesenchymal tumour transmissible by a cell free agent or virus Ellermann and Bang could not, however, emphasise its significance from this point of view, since the neoplastic nature of leukæmia was very doubtful in 1908 Ellermann himself regarded fowl leukæmia as an infectious disease an opinion dictated by his *demonstration of its transmission* by means of virus

On the publication of Ellermann and Bang s reports doubts were immediately raised from various quarters as to whether the pathological pictures observed were really those of leukæmia Skiba (1909) asserted justifiably that the hæmopoietic system of birds is extremely labile and can very easily react with leukæmoid blood pictures in many conditions particularly of an infective character In view of this he questioned the leukæmic nature of Ellermann and Bang s cases Burckhardt (1912) pointed out the very severe leucocytosis (100 000 to 300 000 leucocytes per c mm) occurring in tuberculosis in fowls and supposed that Ellermann s fowl leukosis ' was tuberculosis He emphasised Kasarinoff's (1910) experiments in which leukæmic ' blood pictures were success fully produced by various blood poisons Henschen (1917) assumed that there might be primary anæmias of toxic origin, with secondary implication of the myeloid system

Ellermann (1920) opposed these objections and maintained that there really are leukæmic pathological processes in fowls an opinion which is no longer doubted by other workers During recent years a contrary tendency has asserted itself, namely the occasional classification as fowl leukæmia of pathological pictures which hardly merit inclusion under this heading

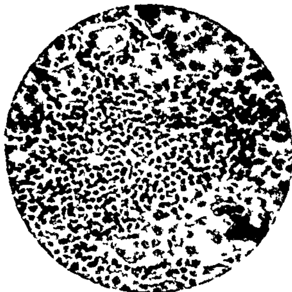


FIG 3.—Interstitial lymphatic infiltration in liver of fowl with leukæmic lymphogenous leukæmia × about 500 (Figs 4 to 6 are from the same case)

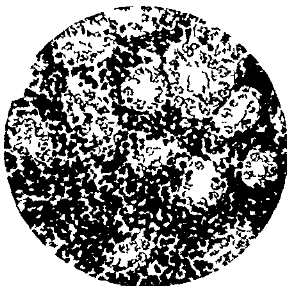


FIG 4.—Interstitial accumulation of lymphocytes in kidney of fowl with leukæmic lymphogenous leukæmia × 500

In the course of many years Ellermann collected a large number of cases of fowl leukæmia occurring spontaneously, and he demonstrated that several types appear with varying frequency. In his first classification (1918) he distinguished these as follows:—

1. Lymphogenous leukæmia ('lymphatic leucosis'), which is divided into an aleukæmic 'extravascular' form with severe, partly nodular, lymphocytic infiltration of the organs, and a leukæmic 'intravascular' form with distinctly leukæmic blood and no nodular infiltrations, but with diffuse intravascular accumulations of large, round, non-granular, basophil cells ('lymphoidocytes') in the organs.

2. Myelogenous leukæmia ('myeloic leucosis'), which may be leukæmic or aleukæmic and is characterised by interstitial myeloid infiltrations, generally consisting of myelocytes, in the organs.

3. Anæmic forms ('leukanæmias'), characterised by pronounced anæmia with many erythroblasts. The reason for including these forms, which are not essentially leukæmic, was because they occurred alternatively with the typical forms of leukæmia in the transmission series and because typical leukæmic pictures resulted from further passages.

Ellermann later (1920) altered this classification, since further research had shown him that the 'intravascular lymphatic leucosis' was not lymphogenous ('lymphatic') and that the cells which proliferated in this form must be considered as basophil erythroblasts (Ellermann's 'erythrogonies'). He reached this conclusion partly from measurements of the mitotic angles in these cells and partly because all conceivable transitions between these cells and mature erythrocytes were found.

Ellermann therefore proposed the following classification:—

1 *Lymphogenous leukæmia* ('Lymphatic leucosis') According to Ellermann this form should always be aleukæmic and should show only more or less nodular lymphatic infiltrations in the organs As will be mentioned later, leukæmic forms are also found, though they are rare

2 *Myelogenous leukæmia* ('Myeloic leucosis') Both aleukæmic and leukæmic forms are recognised in this class Ellermann himself draws attention to the possibility that two types of myelogenous leukæmia may be found in fowls, a *myelocytic* form and a *myeloblastic*

3 *Erythroleukæmia* ('Erythroleucosis') This corresponds to the 'intravascular lymphoid form' in Ellermann's first classification After it had been recognised that this form was characterised by a violent proliferation of basophil and immature erythroblasts, it naturally followed that Ellermann's previous third form 'leukanæmia' should be transferred to the same group, as a special form of erythroleukæmia The relation of these anæmic forms to the pure erythroleukæmias is discussed in greater detail in the next section

Ellermann's last classification has held good in principle The studies of this worker were the impulse which quickened investigations into leukæmia in many different parts of the world, and there is now a considerable amount of material illustrating the occurrence of these diseases, although we are still ignorant of their precise ætiology

VARIETIES OF FOWL LEUKÆMIA

Lymphogenous leukæmia is by far the most frequent form of the spontaneously occurring disease (Ellermann, 1922 Lund, 1926, Mathews and Walkey, 1929, Schaaf, 1936) Like the other forms, it presents only non pathognomonic symptoms — decreased appetite emaciation, and in some cases anæmia, diarrhœa being



Fig 5.—Bone marrow of tibia from fowl with leukæmic lymphogenous leukæmia The marrow consists almost entirely of lymphocytic cells × about 500

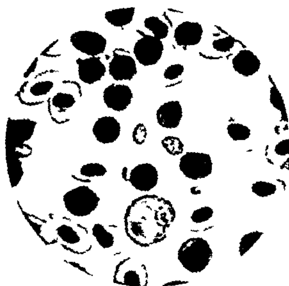


Fig 6.—Blood of fowl with leukæmic lymphogenous leukæmia × about 1500

also observed occasionally Death may, however, occur suddenly, with none of the foregoing symptoms (Ellerman, Mathews and Walkey) Considerable enlargement of the liver is found on autopsy, the weight sometimes being increased ten fold (Figs 1 and 2) The spleen is also found to be enlarged, though to a less degree Fine or coarse whitish grey nodules corresponding to the lymphatic infiltrations can be seen on the surface and on macroscopic section of the organs Infiltrations of a diffuse or nodular character can also be found in the bone marrow, ovaries, intestine, thymus, kidneys, lungs, and heart, whilst the crop, gizzard, testes, thyroid glands, and musculature are often free (Fowls have no lymph nodes, the lymphatic system being represented only by lymphoid follicles, including those in the spleen, marrow liver, intestine, and respiratory tract)

All the affected organs show the same histological change—intense hyperplasia of the lymphatic tissue, a hyperplasia that may be diffuse or nodular The infiltrations consist of small lymphocytic cells, among which a fine meshed fibrillary reticulum can be distinguished The cells not infrequently infiltrate widely into the surrounding tissues (Figs 3 5) Lymphosarcomatous proliferation in the organs has often been described sometimes in association with leukæmic changes, sometimes as isolated lymphosarcomata (see, for instance, Mathews and Walkey, 1929)

It has been mentioned that Ellermann stated that lymphogenous leukæmia in fowls is always aleukæmic Subsequent research has shown that this is not true By far the majority of cases are aleukæmic, though leukæmic cases, often with a large number of leucocytes and with anæmia, have been reported from many quarters In one of the cases described by the present author the blood showed 1 5 million leucocytes per c mm, with 96 per cent of small lymphocytes The

hæmoglobin percentage was 30 the erythrocytes were 1 55 million per c mm (Fig 6)

This form differs from the other types of fowl leukæmia in that it is not similarly transmissible the question of its lack of transmissibility is discussed in detail in Chapter III in which Furth s 'transmissible lymphomatosis' is also considered

Some doubt regarding the definition of lymphogenous leukæmia in fowls has arisen during recent years another pathological picture—'*neuro lymphomatosis gallinarum*' ('fowl paralysis', 'Marek'sche Geflügel lähme', 'range paralysis')—being considered in various quarters to belong to the leukæmia group, for which reason more detailed consideration of this disease is necessary

In 1907 Marek described a disease in fowls, generally fatal and characterised by asymmetrical paralyses iritis (depigmentation of the iris and an abnormal shape of the pupil) shortness of breath, and emaciation The disease is mostly observed in fowls from three to eighteen months of age the fundamental pathological change being an intense infiltration by lymphocytes and plasma cells of the peripheral nerves spinal ganglia and spinal cord In some cases (about 10 per cent) lymphocytic infiltrations are found in various organs, more especially the ovaries and less frequently, the liver spleen, and kidneys Changes are never observed in the bone marrow In the great majority of cases the blood is normal or shows a leucocytosis (Blakemore, 1934 Hepding, 1936) Rarely an increased number of small lymphocytes is observed During the last ten years the disease has increased so greatly in frequency that it is now known in most European countries, the United States of America, Canada Australia, Africa and Japan In many places it occurs with such frequency as to be considered one of the most serious fowl diseases

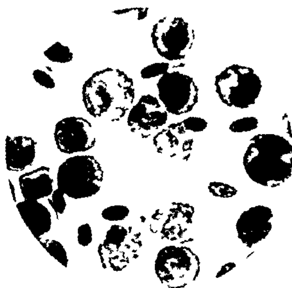


FIG [illegible].—Blood of fowl with myelogenous (myelocytic) leukæmia showing many coarsely granular myelocytes at various stages of differentiation × about 1500

Pappenheimer, Dunn, Cone, and Seidlin published a major work on the disease in 1929, and since then many other studies have appeared. Potel (1939), on the basis of his histological investigations, classified neuro lymphomatosis as belonging to the leukæmias, and Furth (1934*b* and 1935*b*), like Butler and Warren (1938), concluded that 'neuro lymphomatosis is a neoplastic disease allied to (lymphatic) leukosis and sarcoma'. Furth stresses the fact, however, that there are a number of characteristic differences between neuro lymphomatosis and lymphogenous leukæmia, noteworthy among which are the absence from the former of bone marrow changes and the finding that the extension of the disease to internal organs varies, the liver being but rarely affected in neuro lymphomatosis though always in lymphogenous leukæmia. Furth indeed called the latter 'hepato lymphomatosis' to distinguish it from neuro lymphomatosis. Many workers do not share his view, however, that the liver is not commonly involved in the latter disease. Paralysis, which is a cardinal symptom in neuro lymphomatosis, is never observed in lymphogenous leukæmia. Hepding (1936), contrary to Potel, thought that the histological structure of the tumorous formation in neuro lymphomatosis was different from that seen in lymphosarcoma and, generally, in lymphogenous leukæmia. Olson and Dukes (1938) examined the basal metabolism of fowls suffering from neuro lymphomatosis, leukæmia and leukæmic tumours; they found that this was normal in fowls with neuro lymphomatosis but was increased in those with leukæmia and was especially increased in cases of 'lymphocytomata'.

The ætiology of fowl paralysis is not well understood, and one of the main difficulties in investigating it has been the tendency for the disease to occur among untreated experimental fowls bred from the same

parents as those in which the disease has apparently been reproduced The fact that the disease is often enzootic and recurs annually in the same place (Pappenheimer, Dunn, Cone, and Seidlin, 1929) has led to the theory that infection is carried via the fæces and is transmitted through the egg Doyle (1928 29) and Seagar (1933*a*, *b*) observed the occurrence of the disease in chickens hatched from diseased birds Seagar (1933*a*), Hartwigk (1934), Butler, Warren and Hammersland (1938) and many other workers are also of the opinion that neuro lymphomatosis can be transmitted through the egg (according to Doyle, analogously with 'white diarrhœa'), but Asmundson and Biely (1932) claim that it is only a susceptibility which is inherited

Seagar (1933*a*) found the development of typical pathological pictures in a number of fowls which had been fed with extracts of the fæces of diseased fowls Fritzsche (1937 38) stated that he had transmitted the disease by means of fæcal extracts from diseased birds administered to healthy ones by mouth or by injection, but the resulting 'takes' did not show paralysis in a single case They were considered to be 'takes' because it was possible to demonstrate histologically the presence in the nerves of cellular infiltrations, the nature of which it is difficult to determine That the disease is transmissible appears probable from Pappenheimer Dunn, Cone, and Seidlin's (1929) and, especially, Furth's (1935*b*) studies Furth established the fact that the disease can be transmitted by means of cells (lympho cytes), but he did not succeed in demonstrating a cell free agent that can produce the disease in healthy birds In many of the experiments recorded an atypical response followed the inoculation of infective material, for example, Patterson, Wilcke Murray and Henderson (1932) observed cases of erythroleucosis and myeloid and lymphoid leucosis in the groups of fowls they



FIG 8 —Kidney of fowl with myelogenous (myelocytic) leukæmia The renal tubules are separated by enormous infiltrations of myelocytes × about 500



FIG 9 —Bone marrow of tibia from fowl with myelogenous (myelocytic) leukæmia The picture is characterized by massive hyperplastic trabeculæ consisting of myelocytes A compressed marrow sinus runs through the middle of the picture × about 500 (*Cf* Figs 10 and 14)

[P 1

injected and regard all these conditions as expressions of the same transmissible disease It is possible that such results may have been due to the spontaneous development of the leukæmic conditions recorded, and this would indicate the desirability of keeping strains under observation for a few generations before using them for experimental purposes

A series of transmission experiments was carried out by Blakemore (1939) in which the strain of fowls used had been freed of neuro lymphomatosis by inbreeding and rearing the progeny of each generation in isolation The strain was still highly susceptible as shown by the occurrence of the disease following injection of tissues from natural cases and exposure to natural infection In addition to the occurrence of typical lesions of neuro lymphomatosis, some of the experimental fowls became unthrifty and developed atypical lesions in the viscera, especially the heart and liver Passage experiments from the atypically affected fowls were carried out, in the course of which the infective agent gave rise to an acute disease which was inflammatory in nature As in the original experiments, the survivors showed a tendency to develop lymphomatous nodules in the internal organs and nerves, and the author considered that these arose as a result of stimulation by the injected agent While the above results do not give clear support to the theory that the lymphomata are neoplastic in nature, the development of the lesions is in accordance with the findings of Pappenheimer and his colleagues (1929), who, after examining a number of field cases of neuro lymphomatosis, expressed the view that there was undoubted transition between the lesions which histologically appeared to be inflammatory and those which assumed the character of a true neoplasm

It thus appears that the relationship between lym phoid leukæmia and neuro lymphomatosis has not been

explained In both the pathological substrate is a tumorous proliferation of small lymphocytic cells, and yet the pathological pictures show several points of clear cut difference The two conditions present points of similarity clinically as well as anatomically, despite their pronounced and constant differences The question is one of considerable interest, since lymphogenous leukæmia seems to hold a peculiar position among fowl leukæmias, for, as already mentioned, it is not transmissible in the same way as erythroleukæmia and myelogenous leukæmia (see also Chapter III)

Until further information is available it is hardly possible to decide whether neuro lymphomatosis gallinarum is a disease fundamentally different from all the leukæmias or whether neuro lymphomatosis and the so called lymphogenous fowl leukæmia are to be included in one group that is different from myelogenous leukæmia and erythroleukæmia

Myelogenous leukæmia (Ellermann's 'myeloic leucosis') is of rather infrequent occurrence It may be aleukæmic or leukæmic with up to 600,000 leucocytes per c mm The blood picture may be dominated by myelocytes in various stages of development (Fig 7), or there may be a preponderance of typical myeloblasts and, finally, cases occur with large non granular cells with highly indented, segmented nuclei (Ellermann's 'poikilonuclear cells'), reminiscent of Pappenheimer s 'Rieder form' of myeloblasts

The pathological picture is generally characterised by pronounced anæmia (Hb 15 20 per cent), and by more or less pronounced emaciation The course may be acute or prolonged (some months) Autopsy shows enlargement of the liver, but not to so great an extent as in lymphogenous leukæmia, the organ is generally only double its normal size The tissue is either uniformly reddish brown or finely marbled with yellowish

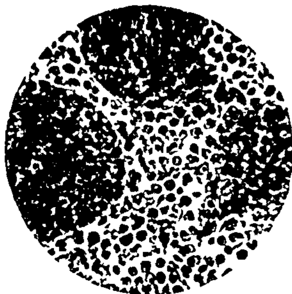


Fig. 10.—Bone marrow of tibia from fowl with mixed myelogenous erythroleukæmia. Slightly dilated sinuses filled with basophil erythroblasts (leukostasis; cf. Figs. 9 and 14) are seen among the hyperplastic trabeculæ, which consist mostly of myelocytes. × about 500.



Fig. 11.—Liver, kidney and spleen of fowl with spontaneous erythroleukæmia. (*Cf.* Figs. 1 and 2.)

veining The spleen is moderately enlarged, with similar colouring The bone marrow is greyish red and of a uniformly firm consistency The microscope reveals myelogenous infiltrations made up of myelocytes or—more rarely—myeloblasts, these are situated peri vascularly in the liver, they occur as a diffuse infiltration in the pulp of the spleen and as dense interstitial masses in the kidneys (Fig 8), where the tubules are widely separated from one another Very considerable hyperplasia of the marrow trabeculæ, consisting of tightly packed myelocytes or myeloblasts, is often observed in the bone marrow The blood sinuses, normally fairly wide are compressed to narrow slits (Fig 9) Besides these changes, considerable accumulations of immature myelogenous cells are found in all the vessels, more especially those of the liver Transitional forms to myelocytomata have been reported, with larger or smaller nodules built up of myelocytes and myeloblasts Mixed cases of this kind were described by Ellermann (1918) Andersen and Bang (1928), Furth (1931c), and Rothe Meyer (1934), while aleukæmic cases of isolated myelocytomata were seen by Teutschländer (1930) and others

Erythroleukæmia (Ellermann's 'erythroleucosis', Engelbreth Holm's 'erythroblastosis') occurs somewhat rarely, but has been observed by most workers on fowl leukæmia This form seems to be peculiar to birds It has been compared with various conditions in mammals and human beings, pernicious anæmia (Ellermann, McGowan), anæmia infantum pseudoleukæmica of von Jaksch Hayem (Henschen, 1917), erythroblastosis congenita (Furth, 1931c, Engelbreth Holm, 1933), but none of these comparisons has been justified The reason for including this disease among the fowl leukæmias is solely that it is so closely connected with myelogenous leukæmia of whose nature there is no doubt, that the

two manifestations are not infrequently found mixed in the same bird (Fig 10) and can be transmitted by one and the same virus (see Chapter III)

When erythroleukæmia is fully developed its course is that of an acute leukæmia which often kills the bird before pronounced symptoms—especially anæmia—have had time to appear Ascites, and petechiæ or ecchymoses in the subcutaneous tissues, are often found on autopsy The most pronounced changes are the great enlargement of the liver and spleen (Fig 11) Both organs present a tense smooth surface, and rupture, with death from hæmorrhage, may occur The colour is a deep reddish violet and the cut surface, like the outer surface, is almost without pattern The kidneys may show similar, though less pronounced, changes The bone marrow may be firm and reddish grey, but it is usually a uniform vivid red and semi liquid ('raspberry jelly')

The blood may show severe anæmia, generally with a very large number of large, round, highly basophil, non granular cells with large, round, leptochromatic nuclei (Ellermann s 'lymphoidocytes') all of which were taken by Ellermann to be basophil erythroblasts or young erythrocytes This opinion has since been confirmed (Furth, Jarmai, Engelbreth Holm) In some cases (Fig 12) these cells are extremely immature ('hæmocytoblasts'), while in others they assume rather the character of erythroblasts with incipient typical erythroblast nuclei and, possibly, even some hæmoglobin in the cytoplasm

Histologically the organs, especially the liver and kidneys, display the change that caused Ellermann to introduce the term 'intravascular lymphoid leukosis' —dense packing of all the capillaries with the cells that predominate in the blood, *i e* basophil erythroblasts or hæmocytoblasts The accumulations of cells are found

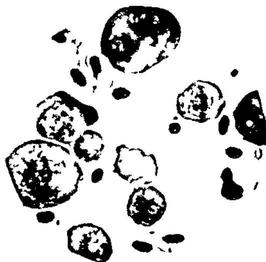


Fig 12 — Blood of fowl with erythroleukæmia Small and large basophil erythroblasts one with two nuclei are seen among the nucleated erythrocytes × about 1,500 (Cf Fig 13)



Fig 13 —Liver of fowl with erythroleukæmia The capillaries between the columns of liver cells are entirely filled with basophil erythroblasts (leukostasis) × about 500

only within the vessels, but they are so enormous that the columns of liver cells often appear to be atrophied (Fig 13) For this excessive intravascular accumulation of cells Ellermann coined the word 'leukostasis' The pulp of the spleen is the site of a correspondingly massive accumulation, so great that the original pattern may be completely lost The trabeculæ in the bone marrow are found to be very fine and compressed between the greatly dilated sinuses containing the tightly packed basophil erythroblasts (Fig 14) In addition to 'pure' erythroleukæmic cases, such as these, others have been described in which the basophil erythroblasts characteristic of the disease form more or less sarcomatous nodules Such cases have been observed, among others, by Andersen and Bang (1928), Rothe Meyer (1934), and Mohos (1939) The nodules were found in the ovaries, skin, liver and kidneys (Fig 16)

Many cases do not display the typical picture, a gradual transition being found to forms in which anæmia is such a prominent symptom that Ellermann placed them in a special class 'leukanæmia' In these cases there is extreme anæmia, with a great decrease in the number of erythrocytes and many polychromatic erythroblasts but few or no true basophil erythroblasts (Fig 15) The organs show only minor changes which, however are of the same kind as those occurring *in* fully developed erythroleukæmia A picture that closely resembles that of erythroleukæmia, with severe 'leukostasis', is often seen in the bone marrow, while it is completely lacking, or is only slightly evident, in the liver, kidneys and spleen The studies of Engelbreth Holm and Rothe Meyer (1932*c*), Jarmai (1932), Stubbs and Furth (1932), and Oberling and Guerin (1934*a*) show clearly that two manifestations of the same morbid process are here involved Engelbreth Holm and Rothe Meyer (1932*c*) considered that anæmic forms

represented the pathological picture which appeared when the erythroleukæmic agent was weakened or the resistance of the host increased Their reason for this was that they observed most cases of anæmia in their group of erythroleukæmias during periods in which the virulence of the virus was relatively weak, as shown by the longer average life of the birds and the relatively large number of cases of spontaneous recovery They explained the fact that the same pathological process can produce fulminant erythroleukæmia when the virus causing the disease is highly virulent, while anæmia occurs when the virulence is weaker, by assuming that the fundamental change in the stem cells forming the erythrocytes, as a result of the influence of the virus, is of a neoplastic nature, with inhibited differentiation as one of its prominent characteristics The result of this process, when it is relatively weak and confined to the marrow, will be a filling of the marrow with immature cells which are unable to differentiate and are thus unable to produce mature erythrocytes This causes anæmia, but no 'leukostasis' in the organs, while a more severe grade of the same process, by attacking the capillaries of the liver and spleen as well as the marrow, will induce so great a production of immature elements that not only are the sinuses of the marrow filled but the whole of the circulatory system is flooded with them, under such conditions the blood picture is that of erythroleukæmia with anæmia and leukostasis is seen in the organs

The anæmic variety of erythroleukæmia has, however, been difficult to distinguish with certainty from other anæmic conditions in fowls It is clear that simple hæmorrhagic anæmias and some of the toxic anæmias are essentially different from erythroleukæmia (Furth, 1931*c*) It was more difficult, however, to differentiate certain forms of anæmia described by Bedson and

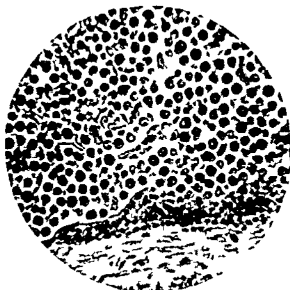


FIG 14.—Bone marrow of tibia from fowl with erythroleukæmia The picture is characterized by enormously dilated sinuses filled with basophil erythroblasts The trabeculæ are reduced to narrow bands especially around the vessel × about 500 (*Cf* Fig 9 and 10)

Knight, and by Bayon. Bedson and Knight (1924) reported eleven cases of anæmia in fowls, characterised by the pronounced yellow colour of the comb, skin, and fatty tissue Many immature erythrocytes were found in the blood, but there was no erythroleukæmia At autopsy, the liver and spleen were found to be moderately enlarged in some cases, while the bone marrow presented a picture that was very reminiscent of that found in erythroleukæmia Bedson and Knight hesitated to conclude that this form of anæmia came under the heading of 'erythroleukæmia' On transmission to healthy animals the development of anæmia or polycythæmia was observed in three out of thirteen cases, but these animals recovered spontaneously

Bayon (1929) described a series of quite similar cases, which he called 'erythromyelosis' A cestode, *Davainea proglottina*, was found in large numbers in the intestine in all the cases Bayon regarded the changes as indicating a disposition to 'infection' by a hypothetical endocellular microbe ('microplasm') Furthermore, he found pronounced osteosclerosis in many of his cases, which he regarded as secondary to the anæmia He explained the apparently paradoxical feature that a great increase in young erythrocytes was found in the marrow although the birds were markedly anæmic, as being due to supercompensatory erythrophagy in the organs, in which he found the content of blood pigment to be increased

Bayon tried to transmit the disease to healthy birds of the same strain (White Leghorns) Four out of twelve died with a similar marrow picture but with an increased number of erythrocytes in the blood A fatal case of progressive anæmia occurred in a later experiment No cestodes were found in the intestines of any of these birds Furth (1931) concluded, from the absence of leukostasis in the organs in these cases of

anæmia, that Bedson and Knight's and Bayon's cases were different from erythroleukæmia According to Furth, the leukostasis in the bone marrow cannot be the decisive factor, since similar pictures may be observed in other toxic anæmias Engelbreth Holm and Rothe Meyer (1932), who observed a similar case of anæmia without leukostasis in the organs in their strains, the transmission of which to healthy birds resulted in typical erythroleukæmia, thought that these 'yellow anæmias' should be regarded as varieties of erythro leukæmia Jarmai (1934) maintained a similar point of view, but Oberling and Guerin (1934) did not venture to include Bedson and Knight's and Bayon's anæmias under the heading 'erythroleukæmic anæmias' McGowan (1926) observed anæmias in birds infected with *Da ainea proglottina* which were similar to those described, and he regarded them as belonging to the leukæmic group Later (1930) however, he changed this opinion, when he suggested that an anæmia different from erythroleukæmic anæmia was caused by *Da ainea proglottina*, on the analogy of *Bothriocephalus* anæmia in human beings McGowan (1930, 1931) found that the injection of liver extracts produced an effect in cases of this anæmia, characterised by an increase in the percentage of hæmoglobin and in the number of erythrocytes, but these extracts did not succeed in curing the condition Generally speaking, the effect does not seem to be very constant or very pronounced In spite of this, McGowan (1932) thought that he had evolved from these experiments a method of quantitative assay for liver extracts

Bayon (1935 36) also tried liver treatment for his 'yellow anæmia' and may have obtained some transient effect He concluded that the experiments 'seem to suggest that the effect of these liver extracts was in some way specific for diseased erythrocytes, but 'a

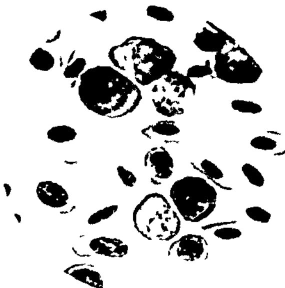


FIG 15 —Blood of fowl with slowly progressing erythroleukaemic anaemia. Basophil erythroblasts are seen, also cells at various stages of differentiation to mature erythrocytes × about 1500 (Cf Fig 12)

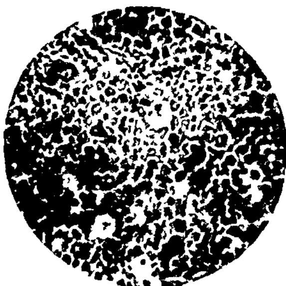


FIG 16 —Sarcomatous nodule in kidney of fowl with erythroleukaemia. The tumour consists of closely packed basophil erythroblasts × about 500

permanent cure did not result' The percentage of hæmoglobin and the number of erythrocytes fell again in spite of continued treatment, and the birds died

It is difficult to disentangle the significance of these observations Liver preparations are certainly of no benefit whatever in definite cases of erythroleukæmia and the erythroleukæmic anæmias (Engelbreth Holm and Rothe Meyer 1932)

The problem of the relation of these anæmias to erythroleukæmia has presumably been brought to a temporary conclusion by Stubbs and Furth's (1932) observation of four spontaneous cases of anæmia in fowls, very similar to those observed by Bedson and Knight and by Bayon, except that there were no parasites in the intestines The tissues of these birds were bright yellow, as in typical cases of 'yellow anæmia' Moreover, pronounced erythroleukæmia and myelogenous leukæmia were produced in some cases by transference of blood from these birds to healthy chickens The reason why Bedson and Knight and Bayon did not obtain similar results may possibly be that their experiments were carried out with a relatively small number of adult birds, which are undoubtedly less susceptible than young chickens

Hence, there can hardly be any doubt of the position of these anæmias in the scheme of classification But that other anæmias of varying ætiology are to be found in fowls is certain It seems probable that infection with *Davainea proglottina* may cause—or contribute to—the development of an anæmia that to some extent reacts to but is not cured by liver preparations

OCCURRENCE AND FREQUENCY OF FOWL LEUKÆMIA

Fowl leukæmia is known in most of the countries of Europe, in America, Australia, Japan, Palestine, and Africa (Bryon, 1929, Schaaf, 1936) Precise information as to the occurrence of the disease, its frequency, and its possible relation to the birds' living conditions, age, breed, etc, is difficult to obtain because no statistical evidence on any large scale is available Furthermore a distinction is not always made in the published reports between leukæmia and neuro lymphomatosis gallinarum, which, as has been mentioned, does not behave in the same way from an epizootic point of view

Most of the information regarding the frequency of fowl leukæmia comes from veterinary institutes, in which records have been compiled of the number of cases found among the birds sent to the particular institute In this way Brieg (1919) found 13 per cent of leukæmias in Denmark, Haupt (1928) 5 per cent in nearly 1700 autopsies in Berlin, Reinhardt (1929) 10 per cent in Leipzig Hare (cited by Furth, 1931) stated that leukæmia was the cause of death in 4 per cent of the fowls in Delaware, Hennepe (cited by Furth, 1931) found it so in 7 per cent of those in Holland Bornstedt (cited by Jarmai, 1934) found leukæmia in 11 per cent of the fowls in Prussia, Lüttschwager (1930 31) in 16 per cent of those in Hanover, and, finally Feldman and Olson (1933, 1934) stated that from 10 to 15 per cent of all fowl deaths in Minnesota were due to leukæmia Schaaf (1936) found lymphogenous leukæmia in from 6 to 7 per cent of adult birds

The disease may thus be said to be of common occurrence Reports have been made from many quarters that fowl leukæmia has been increasing in frequency during recent years Lüttschwager (1930 31) reported an increase from 1925 to 1930 Kitt (1931*b*)

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quotes the following figures from the Tierpathologisches Institut in Munich —

Year	Cases of leukæmia	Autopsies	Per cent
In 1926 there were	17 cases of leukæmia in	251 autopsies	(6.7 per cent)
1927	56	447	(12.7)
1928	74	339	(20.8)
1929	125	456	(26.9)
1930	96	372	(26.6)

and according to Jarmai (1934), the figures have risen similarly, though on a lower scale, in Hungary —

Year	Cases of leukæmia	Autopsies	Per cent
In 1918 24 there were	5 cases of leukæmia in	722 autopsies	(0.7 per cent)
1925 28	6	762	(0.7)
1929	7	304	(2.3)
1930	30	598	(5.0)
1931	24	396	(6.0)
1932	7	157	(4.0)
1933	9	154	(5.8)

Although these figures must be viewed with a certain amount of reserve, it would appear that fowl leukæmia did become more frequent during the years quoted

Strangely enough, Schaaf (1936) found that the figures fell during succeeding years, for in 1930 he observed leukæmia in 9.7 per cent of the fowls examined, in 1931, in 9.3 per cent, in 1932, in 6.2 per cent, in 1933, in 2.6 per cent, and in 1934, in 4.4 per cent

It seems to be generally agreed that the disease occurs most frequently in the winter and spring months and that comparatively few cases occur in the summer months Ellermann (1922) observed most cases from January to April, Jarmai (1930 31) from September to March, Schurmann (1930) from November to February, Kitt (1931) in November, Luttschwager (1930 31) in the winter and spring months Schaaf (1936) found a distinct minimum from June to October as a result of his five years' observations, a finding which was repeated constantly from year to year (Fig 17) According to Schaaf this applies only to lymphogenous leukæmia, erythroleukæmia not being subject to these seasonal variations Lymphogenous leukæmia is by far the

most frequent of the forms of fowl leukæmia which have been described (Ellermann, 1922, Lund, 1926, Mathews and Walkey, 1929, Schaaf, 1936, and others) It is difficult, however, to give definite figures, since many of those quoted in the literature are uncertain It is probable that some of the leukæmias

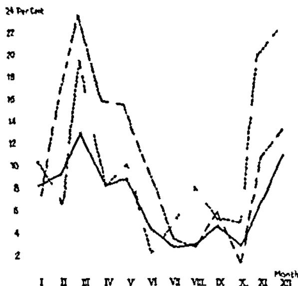


FIG 17 —Percentage incidence of spontaneous lymphogenous fowl leukæmia in Germany during the different months of the year The curves comprise 174 cases

cases in 1930-34
cases in 1930
cases in 1931

(From Schaaf (1936) *Z Infektkr Haustiere* xlix 214)

classified as 'lymphatic' in the older reports were erythroleukæmias (Ellermann's original 'intravascular lymphoid leucosis') and that in some of the more recent reports neuro lymphomatosis gallinarum is included in the group of lymphogenous leukæmia These reservations as regards the exact presentation of the state of affairs do not however alter the fact that lymphogenous leukæmia is the most frequent form

Schaaf (1936) thought he had found a natural explanation of the seasonal variation mentioned above in the fact that the age when leukæmia is most frequently observed is from 9 14 months and that chickens hatched in the spring, constituting the majority of the fowls in most flocks, reach that age between November and May It is not unlikely that this may be the explanation in the case of the observations he made, but the suggestion that there may really be a seasonal variation in the occurrence of the disease gains weight from the fact that a seasonal variation has been demonstrated in the percentage of successes in transmission passages (Engel breth Holm and Rothe Meyer (1935*b*) and see Chap III) In this connection it should also be mentioned that a similar seasonal association with the winter and spring months has been observed for leukæmia in man (Lambin and Gerard, 1934, Engelbreth Holm, 1935) It has not been possible to find a satisfactory explanation of these features Luttschwager (1930 31) observed the occurrence of leukæmia in only one of a number of poultry farms housing a large flock of fowls This particular farm was situated a little higher than the others, on drier and sandy soil The number of cases of leukæmia increased in a dry summer when there was no grass but decreased again next year when the birds had plenty of grass According to this, the relatively small number of leukæmias in summer might be thought to be connected with the protective value of green food, but Engelbreth Holm and Rothe Meyer (1932) were unable, in their experiments, to demonstrate any effect from intensive feeding with greenstuffs It must be emphasised however, that artificially inoculated leuk æmia was employed for these experiments, which there fore cannot be exactly compared with the conditions obtaining in the flocks mentioned above in which the leukæmia was spontaneous

It has also been thought that the fairly regular variation throughout the year might be connected with hormonal conditions and might in particular, be dependent on the egg laying period (*e g* Bayon 1929), but research on the inoculated leukæmias has given little support to this hypothesis (Engelbreth Holm Rothe Meyer and Uhl 1937)

Thus as might have been expected the observations of the apparent connection between fowl leukæmia and the seasons have not led to an understanding of the ætiology of the disease, which is still obscure It is interesting to note that Peacock (1935) found a closely similar seasonal variation in the rate of growth fatality and transmissibility of non filterable fowl sarcomata induced by carcinogenic agents

The transmissibility of fowl leukæmia—which, as has been mentioned does not apply to the lymphogenous form—and the fact that the disease may occur epizootically in large flocks have naturally given rise to the idea (Ellermann and Bang 1908) that it may be contagious Recent research has shown however, that it should rather be regarded as neoplastic, with just as obscure a causal genesis as that of other tumours

That many cases of the disease occur simultaneously in large flocks is not necessarily an expression of its infectious character The explanation of this feature may just as well be that the unknown influence, or influences leading to its development can simultaneously affect many birds living in the same environment

In practice all attempts to infect healthy birds by keeping them with diseased ones (Andersen and Bang 1928 Jarmai 1930 Schaaf 1936) have shown that such infection does not occur Certainly Furth (1931*a*) Jarmai (1932) and Schaaf (1936) have shown that virus can be demonstrated in blood sucking parasites (argasidæ and others) when they have

sucked the blood of diseased birds, but experience has shown that this possibility is of no importance

Patterson *et al* (1932) and Lee *et al* (1937) claim to have observed infection with neuro lymphomatosis, hæmocytoblastosis and lymphoid, erythroid, and myeloid types of leucosis from simple contact, or when the chickens were allowed to grow up on contaminated litter, with the development of all types after infection from one type It is impossible to estimate the accuracy of the diagnoses from the published reports, but the observations conflict with all other experience Patterson and his co workers (1932) mention that their cases of 'myeloid leucosis' might show paralysis This has never been described in cases of real myelogenous leukæmia They also mention that the 'erythroleucotic' cases were decidedly icteric a fact which distinguishes them from typical erythro leukæmia Possibly such 'leucoses' were really examples of the moderate leucocytic infiltrations described by Seagar (1933) and Hepding (1936) in the organs in neuro lymphomatosis gallinarum

Dobberstein (1929), Schurmann (1930), and Schaaf (1936) state that by far the majority of the fowls attacked by leukæmia belong to 'pure' breeds, forced by intensive feeding etc , to produce a maximum of eggs White Leghorns and Barred Rocks (*e g* Feldman and Olson, 1933) seem, in particular, to provide a large contingent of spontaneous fowl leukæmias It is certain, however, that leukæmia is also observed in less 'pure' strains—as a matter of fact inbred 'pure' strains of fowls are not known—and even in the very mixed barndoor fowl (Schaaf, 1936 author s investigations) There seems to be no difference between the sexes as regards susceptibility By far the majority of the cases of the disease occur in adult fowls from one to two years of age but it may also be observed in chickens (Mathews and Walkey, 1929 , Luttschwager, 1930 31), and even in birds which are only a fortnight old

The possibility of a hereditary disposition towards the development of fowl leukæmia has been considered but Jarmai (1935a), after investigating this problem stated that the possibility was very remote Schaaf (1936) came to the same conclusion Wall (1938) stated however, that during the last six years the leukæmic fowls in some of the flocks in Sweden had been excluded from breeding as soon as the disease was recognised with the result that there seemed to be a decrease in the number of cases He could not however give definite figures

There is no doubt that the lymphogenous and myelogenous leukæmias in fowls are diseases of the same nature as leukæmias in mammals Both the blood changes and the changes in the organs are closely similar in each case But apart from these points of agreement, essential differences are found Moreover, one form of fowl leukæmia — erythroleukæmia — is practically speaking, peculiar to birds, a fact that may possibly be explained by the anatomical and physiological differences between the hæmatopoiesis of birds and mammals There exists also one cardinal difference between leukæmia as it occurs in mammals and in fowls namely, that the avian form can be transmitted by a filterable virus This, however applies only to myelogenous leukæmia and erythroleukæmia which are closely connected

The difference between myelogenous fowl leukæmias and mammalian leukæmias, namely the presence of a demonstrable virus in one and its absence from the other is the same as that which exists between virus containing fowl sarcomata and mammalian sarcomata of the same histological type How fundamental this difference may be is not yet known Whether the presence of an easily demonstrable virus in the sarcomatous and leukæmic tissues of fowls means that these diseases are

essentially different from the same diseases in mammals, and whether the relatively easy demonstration of the fowl virus is due to a peculiarity in the mesenchyme of fowls, or to the virus itself, are problems still under discussion More and more investigators seem to incline to the opinion that the viruses demonstrated in fowl tumours of mesenchymal origin do not definitely distinguish such tumours from the corresponding tumours in mammals, but the question cannot be regarded as settled

All these considerations, as has been mentioned before, apply only to myelogenous leukæmia and erythroleukæmia Up to the present no virus has been demonstrated in lymphogenous fowl leukæmia This form seems in no way different from lymphogenous leukæmia in mammals, but it appears in some respects to take an exceptional position in the leukæmic group in birds particularly because, unlike the other forms, it is apparently not transmissible by a virus

In spite of much consideration, in spite of the study of a great amount of material, in spite of numerous observations of the occurrence of the disease in different regions and in varying conditions, the ætiology of fowl leukæmia is still very nearly as obscure as ever Many different hypotheses have been propounded, but we do not know with certainty what factors—exogenous endogenous or both—must exert their actions for the development of the leukæmic processes

CHAPTER II

SPONTANEOUS LEUKÆMIA IN MAMMALS

JUST as leukæmia is known in many kinds of birds and may presumably occur in all of them, so leukæmic conditions have also been found in various kinds of mammals

As has already been mentioned, Leisering (1858) published a case of lymphogenous leukæmia in a horse only a few years after Virchow (1845) had defined leukæmia as an independent disease in man Cases of lymphogenous leukæmia in monkeys were described by Massaglia (1923) and Fox (1923) Siedamgrotzky (1871) Lellmann (1896) and Allen (1901) described cases in cats Fox (1923) in sea lions Finzi (1913) Klügel (1919) Habersang (1924) and others in horses Nartmann (1938) in buffaloes Zschocke (1914) Salomon (1932) and Czymoch (1937 38) in deer (elk roe deer and fallow deer) Lund (1926 27) and Kitt (1931*b*) in sheep Averous (1896), and Krause (1921) in goats Jacob (1908) in elephants Fox (1923) in opossums As none of the reports concerning these animals was anything more than an account of individual cases, they will not be dealt with more fully All the cases seem to have been of lymphogenous type, although myelogenous leukæmia is also said to have occurred in horses (Jarmai, 1934)

More detailed information is available about leukæmia in dogs pigs, cattle, and in particular, rodents, among which mice are of special interest, since the study of leukæmias in mice has contributed essentially to the understanding of the nature of these diseases

LEUKÆMIA IN DOGS

Leukæmia in dogs has been known since Siedamgrotzky's (1871) and Cadiot's (1892) descriptions of cases of leukæmic lymphogenous leukæmia In 1918 Dahlstrom and Henschen were able to collect fifty cases from the literature, and many have been described since The disease does not appear to be particularly frequent Wirth (1931) found only ten cases (0 36 per cent) among 2763 dogs brought to a clinic Only adult animals are attacked (from the age of two and a half years) All breeds seem to be equally susceptible, and most of the recorded cases have occurred in male dogs Share Jones (1927*b*) and Jarmai (1933*b*) noted the development of leukæmia in dogs in apparent connection with traumata All the cases described have been lymphogenous

The lymphogenous leukæmias in dogs have somewhat peculiar features and two different types of the disease occur, one form corresponds to that observed in other animals and in man, the other has the same typical visceral changes but shows changes in the blood that are not typical of lymphogenous leukæmia but rather resemble non specific leucocytosis Both aleukæmic and leukæmic cases—the latter with up to 600,000 white blood corpuscles per c mm —are found in typical lymphogenous leukæmia which is rather rare in dogs

The visceral changes are the same as those found throughout the animal kingdom in this form of leukæmia, *i e* generalised tumours of the lymph nodes, tumours of the spleen, or tumours of the liver, whilst changes seldom take place in the kidneys Lymphocytic infiltrations in the lungs have been described (Bollinger, 1874, Jarmai, 1933) and the lymphatic system of the digestive tract is often involved the

bone marrow also contains numerous lymphocytic cells The histological changes are the same as those seen in other animals and in man

A form which is apparently peculiar to the dog constitutes the majority of the cases in this animal The characteristic pathological feature of this is that, while the visceral changes resemble those of ordinary lymphogenous leukæmia, they are accompanied by anæmia and pronounced leucocytosis, with a preponderance of polynuclear leucocytes and a few myelocytes in the blood Corresponding to this there is a varying myeloid hyperplasia in the bone marrow, in which no lymphogenous tissue such as occurs in 'pure' lymphogenous cases, is found The interpretation of this picture has been difficult, and it has often been taken to indicate leukæmic myelogenous leukæmia The most probable explanation of these strange pathological findings however, has been given in the studies of Dahlström and Henschen (1918) Wirth (1920) and Wirth and Baumann (1933) True lymphogenous leukæmia is undoubtedly involved though with little or no leukæmic change in the marrow the latter showing instead a simple myeloid reaction leading to the leucocytosis In some ways the condition resembles the form of leukæmoid myelogenous reaction observed in animals (particularly mice) which are bearers of malignant tumours of various kinds An explanation of its special features might therefore be found in the fact that the leukæmic processes in these cases—as is observed, for instance, in some cases in man—have not extended to the bone marrow, the latter is thus in a position to react with a leucocytosis that will naturally fail to appear in cases in which the marrow tissue is completely or partly displaced by lymphogenous leukæmic tissue Wirth and Baumann (1933) emphasised that the visceral changes in the two

'types' of lymphogenous leukæmia in dogs are similar as regards lymph nodes, spleen, liver, etc

A 'venereal sarcoma' occurs in dogs, being transmitted from dog to dog by sexual intercourse The condition was regarded as a lymphosarcoma, by de Monbreun and Good pasture (1934), for instance, but this assumption does not seem to be justified on the existing evidence Stubbs and Furth (1934) emphasised that the cells are not lymphocytic they are non differentiated and never mature to lymphocytes, nor do they show any affinity to lymphatic tissue cells Spontaneous generalisation or transportation of the cells in the circulatory system has never been observed Kaalund Jorgensen and Thomsen (1937) *emphasised the similarity to reticulo sarcomata* but did not venture to include the tumours in this group without further investigation

The tumours are most often found subcutaneously on the penis and in the vagina and are easily transmitted experi mentally by direct inoculation or by rubbing into the scarified mucous membrane

LEUKÆMIA IN PIGS

Leukæmia in pigs has been known since 1865 (Leisering) and 1874 (Bollinger) According to Lund (1924) the disease is not of frequent occurrence, possibly because most of the animals are slaughtered while still relatively young Cases have, however, been recorded in quite young animals for instance by Zivero (1904), Niemann (1910) and Biester and McNutt (1926)

Lymphogenous leukæmias and chloromata *in* pigs have been described but myelogenous leukæmias of the usual type are not known The lymphogenous leukæmias (*e g* Raschke, 1915 Lund, 1924, Biester and McNutt, 1926) are generally leukæmic, showing up to 240,000 white blood corpuscles per c mm, with 90 per cent lymphogenous cells The visceral changes are those generally found in this form of leukæmia

Changes are found not only in the spleen and lymph nodes, which may be greatly enlarged, but also in the liver and kidneys, and generally, too, in the bone marrow Microscopically they are typical of lymphogenous leukæmia Aleukæmic cases also appear, but less frequently (Jarmai, 1934) In the course of twenty years among one and a half million pigs intended for slaughter, Manegold and Machens (1927) observed three cases of lymphosarcomatosis with isolated lymphosarcomatous nodes *e g* in the kidneys, and with enlargement of individual groups of lymph nodes No blood changes were reported in these cases

Niemann s (1910) observations on two cases in young pigs seven and ten months old, are of special interest For one thing the youth of the animals is remarkable, and it is also particularly noticeable that the two animals were the offspring of the same boar No conclusion as regards hereditary disposition can, of course, be drawn from an isolated instance such as this, but the observation is of interest in the light of modern investigations of the significance of heredity in the occurrence of leukæmia in mice (see Chapter VI)

Robertson (1909) was the first to observe the condition so extraordinary in many ways which is described as chloroma in pigs About ten cases were subsequently described by Kutsera (1913), Claussen (1928), Hemmert Halswick (1930), and Junack (1930) seven being observed by the last author In every case the discovery was made by chance during the routine examination of the bodies of slaughtered pigs The findings in each case were very similar diffuse, greenish nodes up to 2 cm in diameter in the periosteum and bone marrow of the spine, ribs and other bones and in some cases in the kidneys In addition enlargement of some of the lymph nodes was frequently observed, and the cut surface of these was found to have the same greenish tinge,

which, as also happens in the case of chloroma in man, quickly faded on exposure to the air Microscopic section of these nodes (Kutsera, 1913, Claussen, 1928) revealed a compact mass of myelogenous cells—myelo blasts and myelocytes There are no reports of blood counts *in vivo*, but Robertson (1909) stated that micro scopic examination of clots of blood showed that they contained ' enormously increased leucocytes '

The findings at autopsy thus correspond closely to those in human cases of chloroma, but the fact that all the animals were well nourished and had shown no symptoms of disease before slaughter, when their disease was recognised by chance, represents a very different state of affairs from that occurring in human beings

Another peculiarity of the pathological picture of chloroma is that it is very seldom met with in the higher mammals for—apart from those reported above—only one such case is known, in a cow (Weaver, 1921) In this, the features were the same as those in the pigs, for the animal showed no symptoms and no chloroleukæmic changes were noted until after slaughter

LEUKÆMIA IN CATTLE

Leukæmic conditions are not infrequent in cattle and are known in most countries The disease has been particularly frequent in Germany, more especially in certain parts of the country, where it has caused con siderable loss in a number of herds

The disease has been observed in both sexes The reason that far more cases have been described in cows than in bulls is presumably simply that farmers keep many cows to each bull The animals are generally attacked at the age of six or seven years, but the disease has not infrequently been observed in animals that are a little younger or a little older, according to Czymoch

(1937 38) from three to eight years Leukæmia has been noted in calves, though more rarely Reisinger (1920) observed it in a bull calf eighteen months old, Vogt (1929 30) in a calf a fortnight old, and reports have been made by other authors (see Jarmai, 1934) Katzke (1935) thought he had observed leukæmia in the fœtuses of leukæmic cows, a detailed description was given of only one case, in which a small subcutaneous infiltration was found in an otherwise normal fœtus As no histo logical report is available, the nature of this infiltration is extremely doubtful

Statements concerning the frequency of leukæmia in cattle originate particularly from Germany, where the disease has been subjected to thorough investigation in recent years Dobberstein and Seifried (1938) stated that from 0 15 to 0 4 per cent of all the cattle for slaughter in Germany had to be rejected because of leukæmia These authors also reported that the fre quency of the disease had increased by about 100 per cent during the years 1927 36 Junack (1932) was of the opinion that the disease had doubled or even trebled in twenty five years Lockau (1933) found leukæmia in 0 6 per cent of about 24,000 cattle in Berlin intended for slaughter In agreement with other German authors Schottler and Schottler (1934) reported that the disease was much more frequent east than west of the Elbe and stated that the great increase in the number of cases of leukæmia in German cattle began in the neighbourhood of Memel in East Prussia whence it spread westwards They often observed leukæmia in 10 per cent or more of the herds in the districts most affected By way of illustration, mention was made of four herds in which 9 out of 55, 7 out of 15 12 out of 70 and 16 out of 60 animals respectively had to be killed because of leukæmia

These large numbers were all cases of lymphogenous

leukæmia Sufficient information is not yet available for the proportion of leukæmic to aleukæmic cases to be decided, but both kinds occurred More than 100,000 white blood corpuscles per c mm, with a great increase of lymphocytic elements and often with the appearance of large, non granular, young cells, described as 'lymphoidocytes', with leptochromatic nuclei and nucleoli (du Toit, 1916), were found in the leukæmic cases These cells must presumably be regarded as lymphoblasts In addition, anæmia was often found to be present

An interesting series of investigations was carried out by Knuth and Volkmann (1916), who examined the blood of cows from herds in which leukæmia had appeared They found changes in the blood of eight out of forty animals, in the form of a relative lymphocytosis, with up to 93 per cent lymphocytes, of which some were pathological, resembling those observed in manifest leukæmia Wittstock (1922) followed up the same animals six years later when it appeared that six of the eight had died from leukæmia, while one had been killed on account of pericarditis and only one was healthy

The visceral changes are often characterised by considerable swelling of some groups of lymph nodes with only slight changes or none at all in the liver, spleen and bone marrow This led Knuth and Volkmann (1916) who were the first to give a detailed description of the disease in East Germany, to regard it as an independent disease 'lymphocytomatosis', comparable with Kundrat's leucosarcomatosis Knuth and Volkmann admitted, however, that it could not be definitely distinguished from true leukæmia Later workers (in particular du Toit, 1916) showed clearly that the disease was indeed a typical lymphogenous leukæmia Certainly the enlargement of the lymph nodes is the most common as it is the most



Fig 18—Kidney of bull with aleukæmic lymphogenous leukæmia Infiltrations of lymphocytic cells are seen between the tubules × about 100 (Figs 19 and 20 are from the same case)

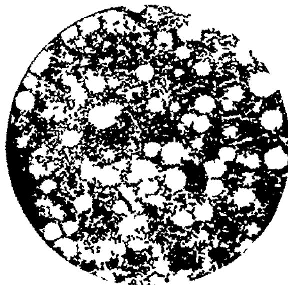


Fig 19—Bone marrow of tibia from bull with aleukæmic lymphogenous leukæmia The tissue of the marrow is over run by numerous lymphocytic cells × about 100

obvious, feature, but typical lymphatic infiltrations are also observed in the heart, lungs digestive tract and—less frequently—the liver and spleen, the latter, according to Junack (1932), is enlarged and the site of leukæmic changes in 25 per cent of the cases More over, tumour like lymphatic infiltrations are often found in the retrobulbar region, leading to exophthalmos, and in the musculature and bone marrow Of particular interest are the descriptions by Neumann (1910), Knuth and Volkmann (1916) and Bulow (1932) of lymphatic infiltrations around the spinal cord, to which, according to Bulow, they have extended from the lymph nodes in front of the spine by spread along the perineural sheaths In a number of cases this has led to paralysis, evidently caused by pressure on the nerves Analogous changes have been observed in cases of lymphogenous leukæmia in mice (see below)

The hyperplasia of the lymph nodes, which is the change most constantly encountered, may affect an individual group or be universal All stages are seen, from moderate hyperplastic enlargement to enormous, greyish white fairly firm, tumour like masses which, when cut not infrequently reveal necrosis

Enlargement of the spleen, though inconstant, may be very considerable—up to about 1 metre in length and 20 kg in weight (Jarmai, 1934) Microscopic examination of the lymph nodes and spleen shows the same diffuse infiltration by pathological lymphocytes as is seen in cases of lymphogenous leukæmia in other species

The liver also may be considerably enlarged though not to the same extent as the spleen Microscopy reveals periportal infiltrations which may be tumour like and nodular and an accumulation of lymphocytic cells in the capillaries This appearance may also be found in livers that show no change macroscopically

Similar changes, diffuse or nodular, are found in the kidneys (Fig 18) The bone marrow has been examined in only a limited number of the published cases It is generally yellowish red and semi transparent, with scattered small red foci, seen under the microscope to be composed of the same lymphocytic cells as characterise the other lesions (Fig 19) A common feature is a considerable swelling of the lymphoid follicles of the digestive tract, and heavy cushion like, submucous infiltrations are usually seen in the wall of the gut The myocardium, and in particular the wall of the auricles, is very often the seat of whitish grey tumour like infiltrations (Fig 20), and similar lesions may in rare cases be observed in the uterus, urinary bladder, udder and lungs

The changes are thus often of a more lymphosarcomatous character than is the case in other animals and in man

Almost every conceivable possibility regarding the ætiology of leukæmia in cattle has been put forward and discussed Knuth and Volkmann (1916) and Czymoch (1937 38) agreed that infection from animal to animal never occurs *The idea that the disease may* possibly be connected with the feeding of cattle is widespread Several authors, *e g* Wittstock (1922) and Czymoch (1937 38), who carried out comparative studies of the conditions of 300 herds in East Prussia, state that the disease is particularly frequent in animals that graze on moist clay or boggy soil du Toit (1920) had come to the same conclusion and compared these conditions with the occurrence of endemic struma in particular districts The observations published do not, however, provide any definite proof that this connection exists

Lockau (1933) found no great increase of the disease at particular seasons but he stated that cases occurred rather more frequently in the October December period



FIG 0.—Myocardium of bull with aleukæmic lymphogenous leukæmia The muscular columns are separated by massive infiltrations × about 100

LEUKÆMIA IN RODENTS

Among rodents true leukæmia has been recorded with certainty only in rats, mice and guinea pigs

McCoy (1914) found no leukæmia or similar condition in 50,000 squirrels in San Francisco and observed only one malignant tumour (sarcoma) in the whole of his material, facts which are in themselves remarkable Fraser (1925), on the other hand, observed a number of cases of lymphosarcomatous lesions (which were transplantable) in the viscera of South American red squirrels Blood changes of a leukæmic character were occasionally observed towards the end in these cases since the tumour cells, which resemble reticular rather than typical lymphogenous cells invade the circulatory system during the last stages of the disease

von Gierke (1914) Schultze (1914), and Zschocke (1914) observed lymphosarcomatous nodes in rabbits von Gierke found a considerable enlargement of the spleen and white nodules in the heart and kidneys in his case In Schultze s case the disease appeared to be transplantable, and a leukæmic condition, with enlarged spleen and in two cases, blood changes, was found among the supposed transmissions

The spontaneous occurrence of true leukæmia has not been observed in wild rabbits, and the fact that only three cases have been described out of the innumerable domesticated rabbits observed for years, in laboratories all over the world shows that the disease occurs only extremely rarely in rabbits

Typical leukæmia has however, been recorded in guinea pigs by Snijders (1926), who—like Miguez (1918)—also described transplantable lymphosarcomata in these animals The leukæmia was transmissible by means of cell containing material (see p 102)

LEUKÆMIA IN RATS

Leukæmia is very rare among wild rats McCoy (1910) and Woolley and Wherry (1911 12) agreed that, on an average, one case of tumour was found in every 1000 of these animals, but that only one lymphosarcoma, which did not show generalised leukæmic changes was observed in every 25 000 animals Bullock and Rohdenburg (1917) also described lymphosarcoma in wild rats

Typical leukæmias both lymphogenous and myelogenous—including cases resembling chloroma—have been observed in laboratory rats Bullock and Curtis (1930) described cases of lymphosarcomata in these animals Wilens and Sproul (1936) found 12 cases of leukæmia of spontaneous origin among 365 animals of an inbred strain of rats, and, of these 12 11 were myelogenous and one was lymphogenous Green colouring of the leukæmic infiltrations was observed in 4 of the 11 myelogenous cases All the cases occurred in animals that were over twenty months old

Rask Nielsen (1938) observed among thirty old rats not of an inbred strain, an animal with a transplantable aleukæmic myeloblastoma of the mesentery with moderate swelling of the mesenteric lymph nodes and in the liver and splenic pulp some perivascular infiltration of similar cells

In a period of five years, Oberling Guerin and Guerin (1939) found 9 cases of leukæmia among 6000 rats 6 of which were lymphogenous and 3 myelogenous These cases, which were very carefully investigated, present many interesting facts The disease was observed only in old animals, at least seventeen or eighteen—and the majority over twenty eight—months of age Of 6 lymphogenous cases 2 were subleukæmic (about 40 000 leucocytes per c mm) and 4 leukæmic with considerable numbers of pathological cells in the blood,

generally atypical lymphocytes with azure granules In one case very young basophil cells resembling lymphoblasts were observed, these should possibly be regarded as hæmocytoblasts ('leucemie a type lympho blastique ou hemocytoblastique') In some cases considerable myelogenous reaction (*cf* lymphogenous leukæmia with leucocytosis in dogs) or anæmia with many basophil erythroblasts in the blood was observed, findings which may make definite classification of these cases difficult

There was very considerable enlargement of the spleen and liver in all the cases, whereas enlargement of the lymph nodes did not occur in every case Micro scopy revealed typical diffuse leukæmic changes in the organs A large tumour of the thymus, filling the anterior mediastinum, was found in one of the sub leukæmic cases

In the 3 myelogenous cases the liver was 2 3 times, and the spleen 10 20 times the normal weight, there was also distinct enlargement of the lymph nodes The blood was examined and found to be leukæmic in 2 of the cases, and in these the leukæmic infiltrations were observed to be green ('leucemie myeloide du type chloro leucemie')

Typical leukæmic infiltrations were seen histologic ally consisting of myelocytic and myeloblastic cells, many of which also filled the capillaries, especially in the liver

Oberling, Guerin and Guerin emphasised the diffi culties encountered in attempts to classify rat (and mouse) leukæmias These are characterised by large immature non granular cells and it is difficult to decide with certainty whether they are lymphogenous or myelo genous They will be further discussed in the section on mouse leukæmia below

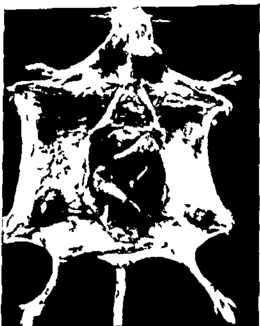


FIG 1—Mouse of inbred strain Aka with leukæmic lymphogenous leukæmia General enlargement of the lymph nodes especially the mesenteric Enlargement of the liver and spleen The intestines have been removed



FIG 2—Mouse of inbred strain Aka with lymphogenous leukæmia General enlargement of the lymph nodes enlarged liver and spleen and considerable enlargement of the thymus gland

LEUKÆMIA IN MICE

As in rats, both lymphogenous and myelogenous types of leukæmia, aleukæmic as well as leukæmic, are known in mice All transitional forms are found, from the classic leukæmic picture through aleukæmic forms to pure neoplastic lymphosarcomata and myelo cytomata or myeloblastomata ('chloromata') One is thus confronted with a series of pathological pictures corresponding so closely to the human leukæmias in their most varied manifestations that it is hardly possible to maintain any longer the sharp distinction between the animal and human disease that has been drawn for many years Until recently there was a tendency to sound a note of warning when an attempt was made to apply conclusions from laboratory observations of leuk æmia in mice to the conditions obtaining in the human disease It has gradually become obvious, however that the leukæmias of man and of lower animals are indeed analogous (see for instance, Furth, Ferris and Rezni koff, 1935) and that, with the investigation of human leukæmia as an end, the study of leukæmia in other species must be amply justified This is particularly true of mouse leukæmia in which the whole clinical and hæmatological picture as seen in man, with all its well known variants—transitions to tumour formation, mono cytic leukæmia, etc —in addition to the two classical forms, is imitated in a way that is really surprising

The first description of leukæmia in mice was given by Eberth (1878) The mice concerned were, like the rats laboratory animals—generally albinos, leukæmia in wild mice has never been described Further cases in laboratory mice were reported by Tyzzer (1907 08) Haaland (1911), von Gierke (1914), Levaditi (1914) and others but it was not until the last eight or ten years that any real advance in the study of the

disease was made Cases were not available for investigation in large numbers until 'pure' inbred strains of mice were employed After a certain number of generations, relatively constant conditions are obtained in the manner in which animals of these strains react to external influences, and one or more diseases which are entirely or partly dependent on heredity are seen to occur with special frequency in many of them The opposite result, that hereditary disease may be made to disappear by suitable inbreeding, can of course also be attained In some of these strains leukæmic processes are very frequent, one form generally predominating

Against the great importance ascribed above to the study of mouse leukæmias, it might perhaps be advanced that the leukæmias now under discussion are not really spontaneous but have been developed in the strain by artificial inbreeding Undoubtedly inbreeding has caused a considerable increase in the number of cases of leukæmia in certain strains, but there is no ground for the assumption that these leukæmias differ in any way from those observed now and then in individual mice which are not inbred, and the theoretical objection to the use of pure strains has in any case, been met by observations with hybrid strains (Gorer, 1938) The inbreeding has not changed the character of the disease to any demonstrable extent but it has provided an enormous amount of experimental material which could hardly have been collected otherwise and which is, in many ways far more valuable than the leukæmias produced by transmission the latter indeed cannot be compared with the spontaneous cases in non inbred strains without certain reservations

The first account of hereditary leukæmia in inbred mice was given by Richter and MacDowell (1929) Lymphogenous leukæmia occurred in a very large number of the animals of a strain (C 58) which had

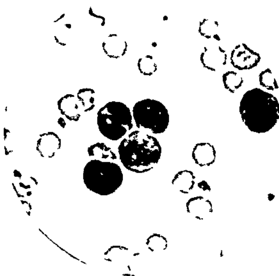


FIG 23 —Blood of mouse (strain Aka) with lymphogenous leukæmia Many large atypical but relatively well differentiated lymphocytes × about 1000

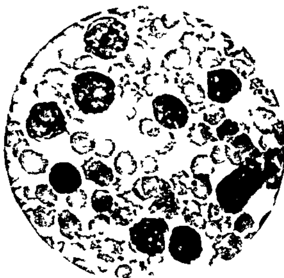


FIG 4 —Blood of mouse (strain Aka) with stem cell leukæmia The picture is characterised by non differentiated cells resembling myeloblasts × about 1000

been inbred since 1921 The disease proved to be transmissible to healthy animals (see below) Ninety per cent of the animals from the 18th to the 23rd generation of this strain developed leukæmia after the age of six months Of 543 such cases of leukæmia, 450 were lymphogenous, 6 were myelogenous and in 87 the type of the disease could not be determined with certainty, generally because of post mortem changes Shortly after this MacDowell and Richter (1931) reported another strain (No 89) in which many of the animals likewise developed leukæmia These also were cases of lymphogenous leukæmia, aleukæmic or leukæmic, with transitions to more tumour like lympho sarcomata

In 1930 Mercier reported 4 spontaneous cases of pulmonary lymphosarcomata in a strain inbred since 1926, and Furth and Strumia (1931) reported a new strain of the same kind Subsequently, Furth, Seibold and Rathbone (1933) and Barnes and Sisman (1939) described this strain and two others, which were called respectively stocks A, R, and S In strain Ak which resulted from the inbreeding of the animals in stock A, lymphogenous leukæmia was common while myelo genous leukæmia was rare In strain Rf which similarly resulted from the inbreeding of stock R, myelogenous leukæmia was common but lymphogenous rare Subse quently both forms (and atypical forms) were found in strain S Finally, Kirschbaum and Strong (1939) reported a strain which had been inbred for many years and in which numerous cases of leukæmia both myelo genous and lymphogenous were observed

Slye s stock is an example of a strain of mice in which the disease is rare since Simonds (1925) found only 39 cases of myelogenous leukæmia 139 cases of lymphogenous leukæmia and 51 cases of lympho sarcoma among the first 15 000 autopsies

These 190 lymphogenous and sarcomatous cases revealed several features of interest Only 28 typical lymphogenous leukæmias were found Subleukæmic conditions were observed in 23 animals and aleukæmic leukæmias in 74 Furthermore, all transitional forms were found from leukæmic or aleukæmic leukæmias to typical lymphosarcomata with or without changes in the blood Simonds described 5 cases which resembled Sternberg's leucosarcomatosis in showing large lympho sarcomata (*e g* in the mesenteric glands), as well as leukæmic changes, he also described 14 subleukæmic cases with lymphosarcomatous visceral changes and, finally, 51 pure lymphosarcomata with no leukæmic changes in the blood or organs

Andersen (1934) found leukæmic diseases in 5 4 per cent of more than 1000 laboratory mice which were not inbred All the cases were lymphogenous, some being leukæmias others lymphosarcomata

It will be understood that it is impossible to give an account of the frequency of occurrence of the various forms of leukæmia in mice generally since our knowledge in this respect is extremely slight The very extensive information which has gradually been collected about the various features of leukæmia in mice originates principally from the artificially inbred strains mentioned above It is, however, definitely established that lymphogenous forms are far more frequent than myelogenous, as was the case in Simonds's and Andersen's material

DIFFERENT FORMS OF MOUSE LEUKÆMIA

Lymphogenous leukæmia in mice is usually characterised by very pronounced visceral changes and considerable general enlargement of the lymph nodes (up to 5 8 mm in diameter) the latter showing a uniformly whitish yellow or yellowish red appearance There is



FIG 25 —Mouse of inbred strain Black with myelogenous leukemia Great enlargement of the liver and spleen but very slight enlargement of the lymph nodes

also pronounced enlargement of the spleen, which may be from 3 to 30 times the normal size often exhibiting visible whitish grey infiltrations of the purple pulp The organ is rigid and smooth The liver may be considerably enlarged, but macroscopic examination generally shows only moderate changes (Fig 21) In many cases a thymic tumour is also found (Fig 22), and this may be so enormous that it fills the thorax and compresses the heart and lungs It is uniformly whitish grey or yellowish on section As already stated, the blood may be leukæmic or aleukæmic, but no information as to the comparative frequency of these states is available except that supplied by Simonds (1925), who, as already mentioned, found 28 leukæmic 74 aleukæmic and 23 subleukæmic cases in 15,000 mice An aleukæmic or subleukæmic blood picture, changing in the last few days of life to a definitely leukæmic picture with as many as 200,000 to 600,000 cells per c mm, is not infrequently seen

The pathological cell which characterises the blood picture of this disease is in some cases a relatively mature though somewhat atypical, small lymphocyte, corresponding to the lymphocytic cell dominant in chronic lymphogenous leukæmia in man (Fig 23) In other cases slightly larger cells with pronounced basophil non granular protoplasm and relatively large, round or often slightly irregular, leptochromatic nuclei containing from two to five nucleoli are found (Fig 24) They do not in themselves bear any resemblance to lymphocytes and doubt has therefore often been expressed about their classification Transitional forms between these cells and typical lymphocytes are however found in some cases and their classification as lymphoblasts is probably justified In other cases, cells are found which are closely similar to these in morphological appearance (*e g* Rask Nielsen 1936), but which now

and then appear to show a positive peroxidase reaction and must therefore be regarded as myelogenous The visceral changes associated with the various blood pictures can hardly be distinguished from one another Whether these forms are to be regarded as examples of stem cell leukæmia, with an accumulation in the blood and organs of a stem cell that may undergo either lymphocytic or myelocytic differentiation (monophyletist interpretation), or whether they are to be regarded as separate lympho blastic and myeloblastic leukæmias, in which however the 'lymphoblast' cannot be distinguished morpho logically from the 'myeloblast' (dualist interpretation), is really of minor importance, no matter how great its theoretical interest To exemplify how difficult it may be to classify cases of this kind in mice—and also in rats —mention may be made of the assertion by Oberling, Guerin and Guerin (1939) that a definite diagnosis may sometimes be impossible ('leucemie a type lympho blastique ou hemocytoblastique')

A spontaneous leukæmia in mice was described by Krebs, Rask Nielsen and Wagner (1930) as 'lympho sarcomatosis' or 'lymphomatosis infiltrans leukæmica et aleukæmica , but Rask Nielsen (1936), in later studies dealing with series of transplantations originating from this case regarded it as myelogenous as did also Kaalund Jorgensen (1936), who characterised the disease as 'myelomatosis (reticulosis)'

Besides these cases of stem cell leukæmia—up to the present presumably the most suitable and non committal term for this form of the disease in mice— there are, as already mentioned, unquestionable cases of both lymphogenous and myelogenous leukæmia, which correspond to typical chronic human leukæmias

Kirschbaum and Strong (1939) emphasised the fact that the stem cells occurring in lymphogenous and myelogenous leukæmia may be quite similar These



Fig 6—Liver of mouse (strain Aka) with lymphogenous leukæmia Massive perivascular infiltration by lymphocytes and many lymphocytic cells in the capillaries × about 100



Fig 27—Lung of mouse (strain Aka) with lymphogenous leukæmia Massive strands of lymphocytes extend from a thymus tumour along the vessels and bronchi into the lung tissue × about 10

authors, who give a detailed cytological description of the various types of cell seen in the two forms of leukæmia, concluded that they are not normal immature cells but are atypical Morphologically, it may hardly be possible to distinguish normal immature cells from leukæmic cells, but the latter are essentially different from normal cells in their tendency to malignant proliferation

Myelogenous leukæmia in mice is best known from the studies of Simonds (1925), and Furth (1934*c* and 1935*a*) and his co workers (Hall and Knocke, 1938, and Barnes and Sisman, 1939) The myelogenous (mainly myelocytic) leukæmias are characterised by very great enlargement of the spleen and liver but, in many cases, only slight enlargement of the lymph nodes (Fig 25) Enlargement of the thymus may occur, as in stem cell leukæmia, and is often very marked The changes in the blood in leukæmic cases resemble those in chronic myelogenous leukæmia in man, with a great increase of myelogenous cells at various stages of differentiation, and a preponderance in some cases of typical myeloblasts, in others of promyelocytes, and in others of myelocytes

Besides these typical cases, instances of chloro leukæmia have been observed (Hall and Knocke 1938) and a case with atypical cells in the blood and visceral infiltrations, of a sort which Barnes and Furth (1937) regarded as megakaryocytic Finally, Furth (1939) has described a case of monocytic leukæmia in a mouse

A common feature of all the forms described is that the histological changes in the organs correspond exactly with those found in leukæmic processes of a similar kind in man The lymphogenous leukæmias are char acterised by diffuse infiltration of the thymus, spleen and lymph nodes by the lymphocytic cells described above or by undifferentiated stem cells The normal

structure is usually entirely obliterated and the histological picture is uniform, with tightly packed small round cells, without granules and with slightly polymorphic rounded nuclei which almost fill the cell A very fine reticulum is seen between the cells The capsules of the lymph nodes are often infiltrated, and the surrounding fat and connective tissue invaded, by leukæmic cells The same cells form perivascular infiltrations in the liver and are found in all layers of the vessel walls A more or less pronounced accumulation of similar cells is seen in the capillaries of the liver (Fig 26)

Both the interstitial infiltrations and the accumulations within the vessels are less constant in the kidneys, but the former are not infrequently found round the pelves of the kidneys, spreading thence to the renal tissue In cases with large tumours of the thymus, infiltrations are often observed to extend downwards to the heart, involving particularly the musculature of the auricles and, farther out, cuffing the pulmonary veins for some way into the pulmonary tissue (Fig 27) Other organs (*e g* the salivary glands) may also be the site of leukæmic infiltration

Paralysis of the hind legs, caused by infiltration around the roots of the nerves and into the spinal cord (Furth, Seibold and Rathbone, 1933), has been observed in mice (Figs 28 and 29), as in cattle

Typical leukæmic infiltrations consisting of myeloblasts, myelocytes and, to a lesser extent, more differentiated cells of the myeloid series are found in the myelogenous forms (Fig 30) These not infrequently spread invasively (*e g* into the muscles) Hæmorrhages are also seen in the lymph nodes and the perinodal tissue According to Barnes and Sisman (1939) it is a significant point in the histological diagnosis that the myelogenous infiltrations contain no erythrogenic foci



Fig 28.—Section of spine of mouse (strain Aka) with lymphogenous leukæmia. Massive epidural lymphocytic infiltration and infiltration of the musculature around the spine. The hind legs were paralysed. × about [illegible]



Fig 9.—Infiltration of lymphocytes round spinal nerves of mouse with lymphogenous leukæmia (strain Aka). The hind leg were paralysed. × about 25

and few, if any, megakaryocytes, these being seen only in the organs in which they are normally found This is important in differentiating myelogenous leukæmia from leukæmoid extramedullary myelopoietic processes, which are not infrequently found in mice in various conditions (see Chapter VIII) As in rats, cattle, fowls and presumably other animals, all imaginable transitions are found, from typical leukæmia with blood changes and diffusely distributed visceral changes, via aleukæmic cases with more tumour like infiltrations in the organs, to pure lymphosarcomata and myelogenous sarcomata, respectively

The part played by mouse leukæmia in recent years, in the study of the leukæmia problem as a whole can hardly be overestimated While observations on leukæmia in pigs and cattle hinted at the importance of the hereditary factor, the examination of strains of mice with and without leukæmia has unmistakably shown the great—though not wholly decisive—significance of this factor The genetic aspect of the leukæmia problem will be dealt with in detail in Chapter VI

It is also principally through investigation of the mouse leukæmias that an insight has been obtained into the interaction of inherited constitutional tendencies and external influences which is generally necessary if the result is to be a manifestation of the leukæmic process These conditions are discussed in Chap VIII

THE SPECIAL POSITION OF LYMPHOGENOUS LEUKÆMIA

It has already been indicated (see pp 29 46) that leukæmia has been observed in many mammalian species If mammalian leukæmia be regarded as a whole, the most remarkable feature is the absolute preponderance of lymphogenous leukæmia and lymphosarcoma

In all the species in which a large number of cases of leukæmia has been observed, the lymphogenous form is predominant None but lymphogenous cases are known in dogs rabbits, squirrels and guinea pigs, and in pigs and cattle, in addition to lymphogenous leucosis, only a few cases of chloroma have been described *Descriptions have been given of undoubted* myelogenous leukæmia and stem cell leukæmia in mice and rats, and of a few cases of monocytic leukæmia and atypical cases in mice, but in these animals too, the lymphogenous forms constitute the vast majority of the cases

The state of affairs found in birds is thus also found in mammals These facts may perhaps indicate that lymphogenous leukæmia occupies an exceptional position among leukæmias This is apparent in the case of birds since the lymphogenous leukæmias, as already mentioned, do not seem, like other forms, to be caused by a virus Furthermore lymphogenous leukæmia in fowls is subject to certain seasonal variations which are not found in respect of the other forms Although similar seasonal variations are not obvious in mammalian or human leukæmias, lymphogenous leukæmia has sufficient peculiar features, of which the significance is not known with any certainty, to justify the suggestion that it may possibly be a unique form of the disease So far as human leukæmias are concerned these features include the fact that about 80 per cent of the leukæmic cases occurring in families are lymphogenous (Petri, 1931 Ardashnikov, 1937, Gottlebe, 1938), as well as the remarkable observation that an association—as yet unexplained—seems to exist between leukæmia and zoster generalisatus, and that about 90 per cent of the cases showing this association are examples of lymphogenous leukæmia (Marques, 1937)

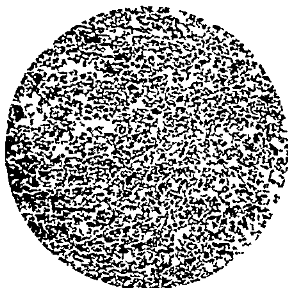


FIG 30.—Liver of mouse (strain Diluted brown) with myelogenous leukæmia. Diffuse and massive cellular accumulations in the capillaries. × about 100

PART II

TRANSMISSION EXPERIMENTS

THE first experiments in which an attempt was made to transmit leukæmia from one animal to another were reported as early as 1872, when Mosler attempted, though unsuccessfully, to produce leukæmia in various animals by the injection of material from leukæmic patients Such experiments have subsequently been repeated many times, but always with negative results, which is not to be wondered at, since modern research has established the fact that the heterologous transplantation of neither leukæmic nor other kinds of neoplastic tissue can be expected to be successful

It is possible to transplant the tissues of leukæmic animals only into animals of the same type In the case of mammals this is further dependent on a close relationship between the animal from which the tissue is taken and that into which it is transplanted Attempts at homologous transplantation will, therefore, often fail if the kind of animal only is considered The first experiment in the homologous transmission of leukæmia was carried out by Bollinger (1874), who injected the splenic tissue of a leukæmic dog emulsified in physiological salt solution, into the lung of another dog, Bollinger chose injection into the lung because this organ had proved to be a site well suited for inoculation in the transmission of tuberculosis Cadiot (1892) attempted to transmit lymphogenous leukæmia to twelve healthy dogs (and also to three cats, two goats, two rabbits and twelve guinea pigs) by subcutaneous, intraperitoneal and intravenous injections of material from a dog suffering from the disease Similar experiments were reported by Émile Weil and Clerc (1904*a*) and others but all gave negative results

Ellermann and Bang in 1908 were the first to succeed in transmitting leukæmia, when they transferred fowl erythroleukæmia and myelogenous leukæmia from diseased to healthy birds Countless experiments with fowl leukæmia have since been carried out all over the world

Attempts have also been made of recent years to transmit other bird leukæmias In 1938 Durant and McDougle tried to transmit the disease from leukæmic canaries to other canaries, by means both of blood and of emulsions of the organs Jarmai (1939) attempted to transmit erythroleukæmia from one budgerigar to twelve others, and also to canaries, goldfinches, fowls, pigeons and mice, but without success Schirrmeister (1938) tried to transmit leukæmia from a turkey to a guinea pig a rabbit and a chicken by the subcutaneous injection of a tissue emulsion A large nodule, the size of a plum, appeared in the chicken at the site of inoculation and this, when examined histologically, displayed a picture identical with that of the infiltrations in the organs of the turkey This finding is of interest in relation to the fact that fowl leukæmia has been transmitted in a few cases to turkeys (see p 65)

In discussing such apparent heterologous transmissions of bird tumours and leukæmias dependent on virus, it is not possible to decide without further data whether an actual infection resulted *i e* whether the virus in question was able to attack the cells of the new host, or whether the case was merely one of simple survival, *e g* of the tissue of the turkey in the body of the chicken, which could hardly be called transmission' to the new species Survival of that nature must be considered rather as a 'tissue culture' of tumour tissue from the turkey in the body of the chicken, and the latter in such cases cannot properly be described as tumour bearing'

CHAPTER III

TRANSMISSION EXPERIMENTS IN FOWL LEUKÆMIA

DURING the years 1908 20 Ellermann and Bang carried out a long series of investigations into 'transmissible fowl leucosis', as Ellermann proposed to call the disease In their very first experiments they established the fact that fowl leukæmia can be transmitted to healthy fowls by means of cell free centrifugates or filtrates, and they assumed that the pathogenic agent must belong to the group of ultravisible viruses

Ellermann and Bang's results were criticised in various quarters and explained in different ways, but they were soon confirmed by other investigators, *e g* Hirschfeld and Jacoby (1909, 1912), Schmeisser (1915) and Magnusson (1915)

After this a long period followed in which interest in transmissible fowl leukæmias appeared to wane, and in which no study of any importance was published on the subject, the interruption of researches, caused by the War of 1914 18, was no doubt, partly responsible for this hiatus It was not until the years 1929 30 that the interesting and important features of these diseases were again thoroughly investigated Almost simultaneously *intensive research was begun in the* U S A by Furth (1929) in Hungary by Jarmai (1929) in Denmark by Engelbreth Holm (1931 32), and in France by Oberling and Guerin (1933) These investigators their co workers and many others have since published numerous studies which have enlarged our knowledge of fowl leukæmias Ellermann's (1920)

results have been confirmed in many respects, in others his conclusions have had to be modified in accordance with the results of more recent research

One of the observations emphasised by Ellermann as remarkable and significant was that during his transmission experiments he observed a tendency to 'change of type' Cases were seen of leukæmia of another type, or of all types, occurring in fowls inoculated with material from one only of the types of fowl leukæmia (lymphogenous, myelogenous or erythroleukæmia), so that the three types might be supposed to be the expression of the influence of one and the same pathological agent This assumption is not compatible with the observations of subsequent investigators, since it appears that the ordinary form of lymphogenous leukæmia is *not* transmissible like myelogenous leukæmia and erythroleukæmia The latter two forms, however, are intimately connected and are not infrequently observed to alternate in the same strain of virus, although 'pure' virus strains also occur Mathews and Walkey (1929) reached the conclusion that lymphogenous leukæmia is not so intimately connected with myelogenous leukæmia, for instance, as Ellermann thought, and Furth in 1931, provided evidence that the lymphogenous leukæmias observed by Ellermann, *as appearing in his series of passages, might in all probability be regarded as examples of spontaneous occurrence* He found 6 cases of lymphogenous leukæmia among 377 experimental birds inoculated with erythroleukæmic material, but 2 cases were also observed among 193 untreated controls None of these cases was capable of further transmission Andersen and Bang (1928) tried to transmit 10 cases of lymphogenous leukæmia, but without success Engelbreth Holm (1931 32) and Jarmai (1932) had the same experience Schaaf (1936)

attempted to transmit 47 cases of lymphogenous leukæmia to a total of 226 animals (including some chickens only a few days old) No takes occurred, but there were 2 spontaneous cases of non transmissible lymphogenous leukæmia Furthermore, all recent investigators agree that among the enormous numbers of experimental birds observed, no more cases of lymphogenous leukæmia in its typical form have ever been noted than might be found in any flock of fowls (Hirschfeld and Jacoby, 1912, Burckhardt, 1912, Schmeisser, 1915, Andersen and Bang 1928, Jarmai, 1932, Feldman and Olson, 1933) Engelbreth Holm did not observe even one case of lymphogenous leukæmia among approximately 7000 birds inoculated with erythroleukæmic substance That not even the 2 per cent or so of spontaneous lymphogenous leukæmia observed in Jarmai's and Schaaf's material was seen is probably due to the fact that most of the birds were young—less than three months old—and that spontaneous leukæmia very rarely occurs in young birds

In fact Ellermann s (1918) series of experiments provides no basis for the assumption that lymphogenous leukæmia is transmissible even with cell containing material As far as can be ascertained from the reports his material contained 8 cases of aleukæmic lymphogenous leukæmia among 373 birds inoculated *i e* about 2 per cent which is no more than might be expected in any flock of fowls Further transmission from these 8 cases when attempted was without result except in one instance [strain E 3rd passage hen N S 75] in which Ellermann reported the observation of three takes—one of lymphogenous leucosis' one of a small solitary 'lymphoma in the liver and one of mixed myelogenous and erythroleukæmia Three months before inoculation with lymphogenous leukæmic material however, the last fowl had been inoculated with blood from an erythroleukæmic case which might quite well explain the positive result while the one lymphogenous leukæmia was most probably spontaneous like the small lymphoma

Ellermann's hen E, from which strain E originated and which was reported in 1918 to be suffering from lymphogenous leukæmia according to a later report (1920) did not suffer from lymphogenous leukæmia but from erythroleukæmia

It must be regarded as an established fact, then, that lymphogenous leukæmia, the course of which, as has been mentioned, is generally aleukæmic and which is the spontaneous fowl leukæmia of most frequent occurrence, is not transmissible and cannot be produced by the viruses that cause myelogenous leukæmia and erythroleukæmia

It should be mentioned in this connection that Furth observed 'lymphomatosis, myelomatosis, and endothelioma caused by a filterable agent' in one of his strains (strain 2, 1934) This observation, however, does not affect the fact that ordinary lymphogenous fowl leukæmia is non transmissible The cases of 'lymphomatosis' observed in Furth's strain 2 were characterised by large, immature cells, described by him (1934*a*) as being 'like large lymphocytes (hemocytoblasts)', which differed distinctly from the relatively far more differentiated small lymphocytes typical of the non transmissible lymphogenous leukæmia As Furth himself pointed out the cell in question seems rather to suggest a non differentiated stem cell The features of this interesting strain will, however, be further discussed in a subsequent section (p 71)

The relation of lymphogenous leukæmia to neurolymphomatosis gallinarum has already been considered (p 9)

Not every spontaneous case of fowl erythroleukæmia or myelogenous leukæmia has been found to be transmissible This may be because only some of the cases are really transmissible or because too few birds have been used for the unsuccessful transmission experiments, or because the birds used have been unsuitable for the

demonstration of transmissibility A fairly large number of birds must be inoculated from each spontaneous case if takes are to be expected, since the percentage of takes is generally only about twenty (Jarmai's strain 1930 was an exception, in that it had a high percentage of takes from the first passage—nearly 100 per cent)

It could hardly be expected that even a very large number of experiments would show that all the spontaneous cases were transmissible, but it is probable that a considerably larger number would have proved so if they had all been constantly transmitted to young chickens instead of to hens, since chickens, as will be mentioned later, are essentially more susceptible than adult birds

Andersen and Bang (1928) found 5 out of 11 spontaneous cases transmissible Jarmai (1932) 3 out of 15, Engelbreth Holm (1933) 5 out of 20 These authors thus found a total of 13 transmissible leukæmias out of 46 spontaneous cases The proportion is analogous with the figures for sarcoma in fowls, from which it appears that only a small number of the spontaneous tumours can be transmitted

STRAINS OF TRANSMISSIBLE FOWL LEUKÆMIA

Most of the 'strains' of transmissible fowl leukæmia which have been described were erythroleukæmias, either pure unmixed as for example the strains of Jarmai (1930 31) Stubbs and Furth (1932), Engelbreth Holm and Rothe Meyer—strain ϕ—(1935*a*), Schaaf (1936) and others, or they were alternately erythro leukæmic and myelogenous (myelocytic) as for instance Furth s strain 1 (1931*a*), Engelbreth Holm s strain R (1932) and the strains of Oberling and Guerin (1934*a*) and others Moreover the erythroleukæmic strains—both pure and mixed—occasionally produce some of the anæmic cases already mentioned These must be

regarded as forms of erythroleukæmia which progress more slowly and occur when the virulence of the agent has decreased or the resistance of the bird, for some reason or other, has increased The course of such cases is generally slower than that of the fully developed erythroleukæmias

In strains in which cases of myelogenous leukæmia are found among the erythroleukæmic cases, the former are generally rare and are found, as for instance in Engelbreth Holm's strain R, largely in the first passages of the strain The course of these myelogenous cases, too, is slower than that of the erythroleukæmic cases Mixed cases with both myelogenous and erythro leukæmic changes are also seen in these strains Finally, strains are known in which all or most of the cases are myelogenous (myeloblastic), *e g* Nyfeldt's strain (1933), which was pure, and Engelbreth Holm and Rothe Meyer's strain T (myelogenic branch), in which about 75 per cent of the cases were myeloblastic and about 25 per cent erythroleukæmic or mixed cases

The connection between these various forms which, in some strains, are caused by the identical virus that in others causes only pure erythroleukæmias (and anæmias) will be discussed more fully later on, when the very interesting connection between fowl leukæmias and the various sarcomatous processes observed in different strains will also be dealt with

The diseases produced by all these apparently different strains can be transmitted to healthy birds by the same method Blood, a suspension of blood corpuscles, plasma, or an emulsion of the organs (liver, spleen, bone marrow, etc) in saline or a similar solution is generally employed for transmission by intravenous, intraperitoneal, intramuscular or subcutaneous injec tion All these methods of inoculation may produce takes, the most certain being the intravenous and the

least certain subcutaneous injection After inoculation the disease will develop in a certain percentage of the birds, which varies with the different strains In attempts to transmit the disease from a spontaneous case the percentage of takes is generally low, though it increases with repeated passage to the maximum characteristic of the strain With Engelbreth Holm s strain R (1933) for instance, the percentage of takes was 15 in the first passage, 18 in the second, 27 in the third, and after the fourth passage 80 100 There are, on the other hand strains—for instance Jarmai's (1930 31)—with which the percentage of takes is about 100 from the very beginning In the same way the period from inoculation to the death of the inoculated bird from leukaemia will often be much longer in the first passage than in the later ones For the strain R mentioned above this period was about 170 days in the first passage, while in the eighth and subsequent passages it was only from 10 to 14 days The figures, however, show considerable variation with the different strains

During the first five to ten passages the percent age of takes usually increases to a maximum where it remains A constant increase in virulence beyond the first passages indicated by a shortened course of the disease, has, however, also been observed, *e g* in Jarmai's strain (1938) in which the average period of life for the inoculated birds fell from 20 to 11 days in the course of eight years

Transmission can be achieved with remarkably small doses Furth (1932*b*), for example, obtained takes of erythroleukaemia with 0 000001 c cm of plasma and 0 00001 c cm of cell suspension while Engelbreth Holm (1932) observed takes with 0 00001 c cm of blood When such small doses are used, the percentage of takes will generally be lower and the life of the birds longer It is also a general experience (*e g* Rothe

Meyer and Engelbreth Holm, 1933*a*) that transmission is less certain when plasma is used than when blood or a suspension of blood corpuscles is employed It must therefore be assumed that leukæmic blood cells contain greater concentrations of virus than does the plasma

VARIATIONS IN PERCENTAGE OF TAKES

While the percentage of takes and the rapidity of development of the disease are thus to a certain extent dependent on the substance used and on the method of inoculation (the most successful results are undoubtedly obtained by the intravenous inoculation of blood in adequate quantities, for instance 1 c cm), both these factors are influenced also by the age of the bird inoculated Engelbreth Holm and Rothe Meyer (1932*a*) demonstrated that far more takes resulted from trans mission of erythroleukæmia to chickens only a few days old than from transmission to adult birds The typical pathological picture was also successfully reproduced by the injection of erythroleukæmic blood into fowl embryos in the egg (Engelbreth Holm and Rothe Meyer, 1932*a* , Jarmai, 1933) In conformity with this, Stubbs and Furth (1930 31) found that hens over two years old were less susceptible than those under one year

Other factors than the inoculating substance, the method of inoculation and the age of the birds used, may, however, influence the frequency and strength of the takes There does not seem to be any great differ ence in the susceptibility of the various kinds of fowls to inoculation (*cf* Stubbs and Furth, 1930 31 , Schaaf, 1936 , Stubbs, 1938) Both sexes seem to be equally susceptible

While all the methods of transmission by which leukæmic material is introduced into the bodies of healthy birds by incision or injection have proved

successful, feeding with virulent material has been shown in many experiments to be incapable of transmitting the disease (Andersen and Bang, 1928, Engelbreth Holm, 1933, Schaaf, 1936)

Just as the spontaneous occurrence of fowl leukæmia mentioned in Chapter I, seems to be subject to seasonal variations, so the susceptibility of fowls to erythro leukæmia after inoculation also varies during different

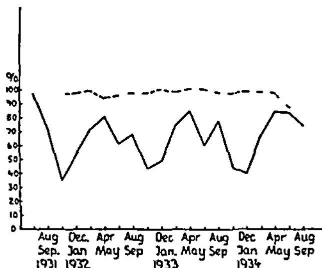


FIG 31 —Fluctuations over three years in the percentage of takes after intravenous inoculation of hens and chickens with erythroleukæmic blood The material comprised about 1000 chickens (--) and 1000 hens (———) of three different strains (R T_1 E–S) (From Engelbreth Holm and Rothe Meyer (1935) *Acta path Scand* xii 366)

seasons Over a period of three years Engelbreth Holm and Rothe Meyer (1935) found uniform variations in susceptibility to three different erythroleukæmic strains As appears from Fig 31 these variations were found only in adult fowls (over six months old), whereas chickens (under three months old) showed the same susceptibility all the year round and were always more susceptible than adults The greatest susceptibility in

adult fowls was observed from April to May and the least from October to December The decrease in susceptibility to inoculated leukæmic virus during the autumn months was indicated not only by a decreased percentage of takes but also by a prolongation of the average period of life of the birds after inoculation The takes in the autumn also occurred comparatively frequently as erythroleukæmic anæmia and not as fully developed erythroleukæmia, a fact which seems further to support the assumption that this erythroleukæmic anæmia (see p 16) is a weak manifestation of erythro leukæmia Moreover, far more spontaneous recoveries were seen in those periods in which the susceptibility of the fowls was relatively slight In agreement with this, anæmic cases or spontaneous recoveries are seldom, if ever, found in young chickens, whose susceptibility is not subject to seasonal variations

It was mentioned in Chapter I that there is no satisfactory explanation of the seasonal variations, but there are two possibilities which might *ex hypothesi* be considered in this connection, firstly, the variations might conceivably be due to seasonal differences in diet, and here attention has been especially directed to greenstuffs, secondly, fluctuations in the hormonal milieu of the birds might reasonably be expected to account for the peculiar difference between quite young and adult birds Experi mental observations however, have so far lent no support to either of these hypotheses (Engelbreth Holm, Rothe Meyer and Uhl 1932 1937, Schaaf, 1936) The findings regarding these seasonal variations in suscepti bility to inoculation with virus material may be compared with those of Peacock (1935) concerning susceptibility to transplanted fowl tumours (p 25)

SPECIFICITY OF FOWL LEUKÆMIA FOR SPECIES

Up to a few years ago the virus of fowl leukæmia was considered to be highly specific for species, since all attempts to transmit the disease to other closely related kinds of birds had given negative results In 1932, however, Engelbreth Holm and Rothe Meyer reported that they had successfully transmitted fowl leukæmia to guinea fowls, three out of ten guinea fowls having died from erythroleukæmic anæmia after the intravenous injection of blood from erythroleukæmic fowls These experiments were subsequently confirmed by Jarmai (1935*a*) and by Schaaf (1936) who by successful reinoculation from guinea fowls to fowls, proved that the disease in the two kindred species of bird was the same

In 1933 Stubbs transmitted fowl leukæmia to a fowl pheasant hybrid, and Jarmai (1935*a*) carried out successful transmission to pheasants, from which the disease was retransmitted to fowls Jarmai's experiments, in which the disease was successfully transmitted to turkeys and further through turkeys for some passages are of special interest On retransmission from six turkeys to twenty one fowls, only one take resulted, which may indicate that in its passages through turkeys the virus had become less virulent for fowls, a fact in accordance with experience of other kinds of viruses

Although the virus of fowl leukæmia is thus not strictly species specific it nevertheless remains true that the disease can be transmitted only to kindred species of fowls, *i e* kinds of birds so closely related to fowls that cross breeds between them and fowls are known In spite of numerous attempts in various quarters, the disease has never been successfully transmitted to more remote kinds of birds, such as quails peacocks ducks, geese canaries and others

DEVELOPMENT OF LEUKÆMIC PROCESSES AFTER TRANSMISSION

The leukæmic processes developing in the body of a fowl after inoculation with leukæmic substance are probably nearly always caused by the action, on the blood forming tissues, of the virus contained in the substance inoculated

In certain cases, however, another process may also occur Crank and Furth (1931) pointed out that when large quantities of leukæmic blood were injected into the vascular system of a healthy bird, the inoculated cells continued to multiply and, in the course of a couple of days, proliferated to such an extent that the blood became completely leukæmic and the bird died A large number of pathological blood cells were certainly found in the capillaries of the organs but, apart from this, there were no leukæmic changes in the bone marrow, for instance which consisted largely of fatty tissue The direct transplantation of cells, similar to the trans plantation of tumours in mammals, is here involved It should be noted that the visceral changes in these birds differ essentially from those observed in birds developing leukæmia after the injection of cell free substance since in the latter the pathological picture is due to proliferation of the host's own hæmopoietic cells, which are transformed into leukæmic tissue by the action of the inoculated virus

Fowl leukæmia transmitted by the ' cell free ' method thus differs fundamentally from the transplanted mam malian leukæmias or tumours, whose pathological picture is produced by a multiplication of cells foreign to the host

The virus soon disappears from the vascular system of fowls after the intravenous injection of virus containing material, *i e* it soon becomes impossible to demonstrate its presence by injecting the blood into other fowls

Crank and Furth (1931) found that blood removed from inoculated fowls even as early as thirty minutes after inoculation would not infect others In Rothe Meyer and Engelbreth-Holm's (1933*a*) experiments, how ever, the virus did not disappear from the vascular system until twenty four hours later After a few days (about two to fifteen depending on the virulence of the particular virus) the blood of the host again becomes infective and, simultaneously, the first patho logical cells can be demonstrated These observa tions have been confirmed by Ruffilli (1938*c*, *e*) and by Storti and Brotto (1938), who also investigated the virus content of the organs Although Ruffilli (1938*c*) was of opinion that the virus disappeared from the blood shortly after inoculation he did not think that it vanished entirely from the blood corpuscles These authors found that virus could be demonstrated continuously in the bone marrow from shortly after inoculation until the bird died from leukæmia, the other organs did not contain the virus so regularly during the period in which the blood was virus free They therefore con cluded that the virus is fixed in the bone marrow, and possibly in the spleen and liver The bone marrow is also the first organ in which leukæmic changes can be demon strated a fact established by many investigators (Kogler, 1932 Engelbreth Holm, 1932 and others) The characteristic pathological changes do not appear in the liver, spleen and other organs until after the occurrence of changes in the blood (Engelbreth Holm, 1932 1933) Storti and Brotto (1938) thought that they were justified in concluding from these features in conjunction with the demonstration of the fact that the virus is first and foremost fixed in the bone marrow, that the leukæmic changes in such organs as the liver and spleen arise from colonisation' by cells originating in the marrow, and not from a virus induced change in the hæmopoietic

cells of those organs themselves Nevertheless, the actual experiments by Storti and Brotto, in which the inoculated virus was found to be taken up by the bone marrow of some bones and, though less regularly, by the spleen and liver, seem to conform equally well with the view that during the action of the virus all parts of the hæmopoietic system acquire a leukæmic tendency, since the bone marrow, at any rate, reacts as a whole to the virus The reason why the spleen and liver take up the virus less regularly may indeed be purely quantitative, since these organs contain far fewer susceptible cells than the marrow

Previous to this, Engelbreth Holm's (1933) examination of the liver tissue of fowls at various periods after inoculation had led him to the conclusion that an autochthonous production of cells probably takes place in the liver, since small islands of hæmocytoblasts are found in the sinusoidal spaces of the liver capillaries, some of them in mitotic cell division, at a time when the blood contains only a few pathological cells Storti and de Filippi's (1937) histological examination of the liver, however, did *not provide any basis for* such an assumption, and it is perhaps hardly possible to solve this problem conclusively by morphological investigation alone It is one of fundamental importance, since it involves the question whether leukæmia in fowls should be regarded as a pathological process in one *individual* organ, possibly in a single cell area of that organ—the bone marrow—or whether it should be considered as the expression of an extensive change of all the elements of a whole system There is support for both points of view in respect of fowl leukæmia, and the same considerations apply in the case of the mammalian leukæmias

SPONTANEOUS REMISSION AND RECOVERY

It has been mentioned above that spontaneous remission and recovery have sometimes been observed in fowls after their successful inoculation with leukæmic material When studying the fate of inoculated virus in these birds, Rothe Meyer and Engelbreth Holm (1933*a*) found three cases in which the blood was at no time leukæmic after the virus, as mentioned above, had disappeared 24 hours after inoculation, and had again been demonstrated eight days later After the virus had disappeared from the blood for the second time, the birds, like those which became leukæmic and recovered spontaneously remained refractory to subsequent attempts at transmission This observation is significant since it indicates that birds which have been previously inoculated with leukæmic substance, and have not reacted to it by developing leukæmia, should not be used for further transmission experiments with leukæmia, as they may quite well have acquired the same 'immunity' as is found in birds which have recovered spontaneously from the disease On the other hand, birds which have proved refractory to several inoculations, may in rare cases react to a subsequent 'transmission by developing leukæmia, thus indicating that the immunity acquired may be only relative or temporary Rothe Meyer Engelbreth Holm and Uhl (1935) found that one out of eleven fowls which had recovered spontaneously was refractory to nine subsequent re inoculations but died of leukæmia after the tenth

Observations of the virus content of the blood of birds that are thus 'immune' after inoculation show that the injected virus rapidly disappears from the circulation and cannot be demonstrated again later Investigations of the immunity of birds which have recovered spontaneously will be discussed in Chapter IV

That a varying number of inoculated fowls can recover spontaneously, *i e* that the leukæmic processes acquired by inoculation can regress, does not necessarily imply that leukæmia is a less malignant disease in fowls than in mammals It must be remembered that the cases in question were not spontaneous but were produced by inoculation, although it is worthy of note that the changes originated from the tissues of the birds them selves and not from the introduction of foreign cells, as in the case of leukæmias transplanted into mammals This can hardly indicate anything but that the co operation of a number of factors is necessary for the manifestation of progressive leukæmic change, the virus being only one of these factors and, by itself, insufficient We are ignorant of the nature of the other factors The genetic constitution of the birds may possibly play a part, as in mouse leukæmia

THE RELATION BETWEEN FOWL LEUKÆMIA AND SARCOMA

It has already been indicated that there are strains in which fowl leukæmia occurs in intimate connection with sarcomata of different kinds In 1933 such strains were reported almost simultaneously from France, the U S A and Denmark

Oberling and Guerin (1933*b*) attempted to produce tumours in a hitherto pure erythroleukæmic strain by previously treating the tissues of the inoculated birds with kieselguhr, embryonic tissue and thorotrast, but they were unsuccessful It appeared, however, that inoculation with blood or an emulsion of organs from this strain which had been kept in glycerine in an ice box for some days (6 to 47) produced, in addition to erythroleukæmia, some cases of spindle cell sarcoma or endothelial sarcomatous change These tumours could be trans planted into healthy birds, but the takes were always

accompanied by the development of leukæmia Oberling and Guerin assumed that this indicated that the virus was modified by keeping ('mutation de virus'), so that it was then able to influence both hæmopoietic cells and mesenchymal cells of other kinds Their results were later confirmed by Troisier (1935)

Oberling and Guérin found also carcinomata, considered to be dependent on the same virus in two birds Nothing corresponding to these cases has been observed in other strains, and the observation is exceptional in respect of the enormous amount of the total material examined The supposed connection between these carcinomatous processes and leukæmia sarcoma virus may therefore be doubted

The second combined' leukæmic sarcomatous strain was observed by Furth (1933) It originated from a chicken which died from leukæmia of a peculiar type the blood containing 130,000 white blood corpuscles per c mm mostly large immature lymphoid cells—cells like large lymphocytes (hemocytoblasts)' As mentioned before, these cells do not correspond to the smaller more differentiated lymphocytes which characterise the picture in the non transmissible lymphogenous fowl leukæmias Many white nodes up to 1 cm in diameter were found in the liver, spleen, heart and kidneys These consisted of the same kind of cells as those seen in the blood

Transmission from this bird resulted in 'strain 2', which caused alternating cases of 'lymphomatosis (hemocytoblastosis) —as in the original bird—of myelogenous leukæmia and of endotheliomata, the tumours not being observed without simultaneous leukæmia It appears from Furth s investigations that these various processes may be attributed to a single virus which causes a tumor like multiplication of mesenchyme or endothelium These adherent, or perhaps syncytial,

cells either remain undifferentiated or they become detached and transformed into cells like large lympho cytes (hemocytoblasts)"

Finally, Rothe Meyer and Engelbreth Holm (1933*b*, 1935*a*) reported a third strain (strain E S) of a similar kind It originated in a chicken with myelogenous (myelocytic) leukæmia, with multiple myelocytomata in the kidneys and liver, and spindle cell sarcomata in the musculature of both legs

When the blood was inoculated erythroleukæmia was produced, and the sarcomata were transplantable to healthy birds This case looked as though it were one of a chance simultaneous occurrence of leukæmia and sarcoma But even in the third passage erythro leukæmia occurred in the birds into which the sarcoma had been transplanted, and sarcomatous changes de veloped in a number of the birds which had been injected intravenously with the erythroleukæmic blood Rothe Meyer's (1934) thorough investigation of this strain revealed that a single virus might be considered to be the cause of both the leukæmia and the sarcoma Intra venous injection generally resulted in leukæmia (Fig 32), and intramuscular or subcutaneous inoculation gener ally produced sarcoma A sarcoma was, for instance, not infrequently seen at the site of inoculation (Fig 33) Rothe Meyer utilised this feature in trying to separate the leukæmic and the sarcomatous viruses, on the assumption that they were different Unmixed erythroleukæmia resulted from forty three intravenous passages After this, however, the virus was still able to produce sarcoma when injected subcutaneously Similarly, twenty eight passages of pure sarcomata were effected by transplanta tion, after which intravenous injection of the virus still resulted in erythroleukæmia

This strain, like Furth's, differs from Oberling and Guerin's in that its ability to produce tumours is not



FIG 3 Blood of fowl with erythroleukæmia produced by intravenous injection of blood from a mixed sarcoma erythroleukæmic strain (E–S) Three mitoses in basophil erythroblasts are seen ×900 (*Cf* Figs 34 36)



FIG 33 —Wing of fowl with sarcoma at site of intravenous inoculation of erythroleukæmic blood from a mixed sarcoma erythroleukæmia strain (E–S) (*Cf* Figs 3 34 and 35 Figs 33 to 35 are from Rothe Meyer (1934) *Experimentelle studier over forholdet mellem leukose og sarcom hos Hons* Copenhagen)

[p 7

dependent on some antecedent interference (freezing, storage, etc), the virus in the blood of a bird suffering from erythroleukæmia being able to cause the development of sarcoma directly, and *vice versa* While the majority of the cases observed with this strain were mixed, the fact that each of the two pathological pictures, leukæmia and sarcoma, could be produced in a relatively 'pure' state is one of the respects in which it differs from Furth's The sarcomata that occur with this strain are generally endotheliomatous (Fig 34), or angio endotheliomatous, or, more rarely, spindle cell sarcomata (Fig 35) as in the original bird Rothe Meyer observed every conceivable morphological transition from endotheliosarcomatous elements to large free hæmocytoblasts

Stubbs and Furth (1935) reported a very similar strain (strain 13), which originated from a bird in their 'pure' erythroleukæmic strain (strain 1) and in which a sarcoma occurred at the site of inoculation after intravenous inoculation with blood in the usual way With this strain the tumours observed were also endotheliomatous but they were more widely distributed than those with Rothe Meyer and Engelbreth Holm s (strain E S) Stubbs and Furth were unable to separate a leukæmic from a sarcomatous virus, in agreement with Rothe Meyer, they concluded that the various manifestations were "caused by a single virus with ability to produce diffuse endothelial growth and erythroblastic proliferation" Extensive hæmorrhage was frequently found in the organs with both strains, apparently caused by small angio endotheliomatous foci Furth (1936*a*) investigated a third 'mixed strain (strain 12) It originated from a chicken inoculated intravenously with strain 2—'lymphomatosis (hemocytoblastosis)'—which developed a transmissible osteochondrosarcoma In this strain, however, he succeeded

in showing that in all probability there was a mixture of two viruses, since he was able to re isolate 'strain 2 virus' from it, this was possible because the osteo chondrosarcoma virus of strain 12 became effective only when it was applied to bone Furth (1936*b*) pro duced an artificial 'mixed' strain by mixing leukæmic virus (strain 1, erythroleukæmia) and 'pure' sarcomatous virus (strain 11, spindle cell sarcoma, resembling Rous's sarcoma I) In this mixed 'strain' the two viruses preserved their characteristics unchanged when injected into the same bird and were relatively easy to re isolate

After the discovery of these various leukæmic sarcomatous strains, the leukæmic strains which had been considered as 'pure' were closely examined by many workers in order to find out if, under suitable conditions, they could produce true sarcomata similar to those caused by Oberling and Guerin's strain described above This proved to be so as regards a fair number of strains of fowl leukæmia

Jarmai (1935*a*) for instance, found small tumour like infiltrations with his 'pure' erythroleukæmic strain after intramuscular transplantation of small fragments of the leukæmic organs, they were capable of further transplantation but could not be isolated from the simultaneous leukæmic changes, which killed the birds so quickly that the resulting sarcomata never reached any great size The tumours observed were partly spindle celled and partly round celled

In 1936 Uhl Engelbreth Holm and Rothe Meyer published similar investigations of their 'pure' leukæmic strains 'R' and 'T' Small nodes with the histological structure of fibrosarcomata, myxosarcomata or angio endotheliomata occasionally occurred with both these strains after subcutaneous inoculation with the leukæmic blood The injection of normal blood never produced such changes Transplantation of these nodes was



Fig 34 Endothelial sarcoma in kidney of fowl after intravenous injection of blood from a mixed sarcoma-erythroleukæmia strain (E-8). Coherent large atypical endothelial cells with transition to basophil erythroblasts are seen × about oo (*Cf* Figs 33 and 35)

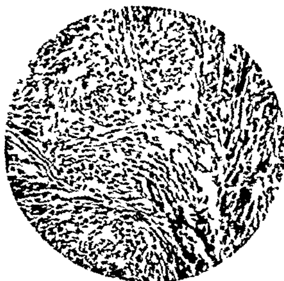
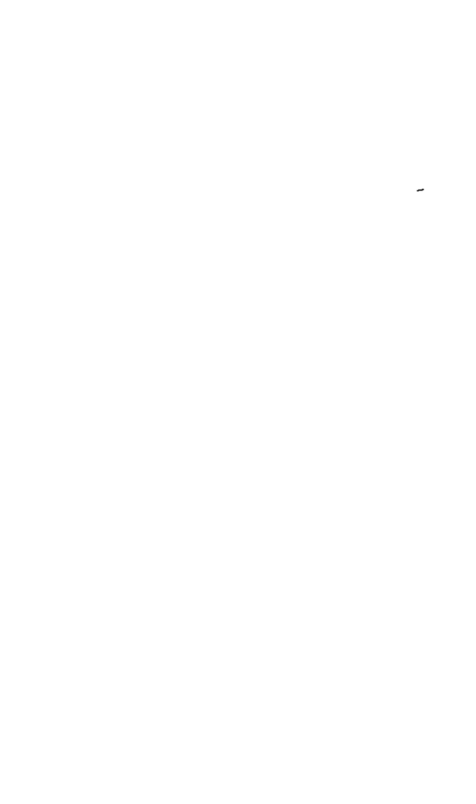


Fig 35—Spindle-cell sarcoma of fowl after intramuscular injection of blood from a mixed sarcoma-erythroleukæmia strain (E-8) × about oo (*Cf* Figs 33 and 34)



successful in the case of strain T—in which the leukæmic cells were immature hæmocytoblasts which in some cases differentiated to myeloblasts, in others to basophil erythroblasts—but not in strain R, whose characteristic cell was a relatively more differentiated hæmocytoblast or erythroblast. Storti and Zuetta (1938) also observed the development of more or less polymorphic sarcomata in similar experiments.

Oberling and Guérin (1938*a*) in extensive research on these problems found that the sarcoma-producing ability of their erythroleukæmic virus was particularly marked in the case of intracutaneous inoculation with small quantities of diluted blood, which produced fibro-myxosarcomata.

Not every strain of fowl leukæmia virus, however, can produce sarcomata as described. Stubbs (1938) for instance, in spite of several attempts, never managed to produce sarcomata from his erythroleukæmic strain by intramuscular or subcutaneous inoculation. Mohos's (1939) erythroleukæmic strain, too, was unable to produce sarcoma, even with preceding glycerination as in Oberling and Guérin's first experiments. It is curious that this strain originated from a bird with a pronounced aleukæmic 'erythrogonio-sarcoma' in the liver.

INTERRELATION OF THE DIFFERENT FORMS OF FOWL LEUKÆMIA

Reports are thus available of carefully investigated strains of virus which can produce only erythroleukæmia (and sometimes myelogenous leukæmia, which is intimately associated with it) and of other strains that produce the same kind of leukæmia and also sarcomatous proliferation of more or less organised and differentiated endothelial elements or fibrocytic mesenchymal derivatives. As has been emphasised by Foulds (1934) these strains seem to form a series via the Murray-Begg

other is that, although the virus has a special affinity for one definite type of cell at a certain stage of differentiation, it is also capable, though to a lesser degree, of attacking the more differentiated descendants of this cell, as well as its less differentiated ancestors

According to the first theory, the various pathological pictures seen in a strain caused by a certain virus are to be explained as an expression of the differentiating possibilities of the cell specifically sensitive to the virus If this cell be an erythrocytic stem cell for instance, so far differentiated that it can be further differentiated only to erythroblasts, the result of the transmission passages will be erythroleukæmia only Similarly, the virus of the Rous sarcoma must be specifically adjusted to a relatively well differentiated cell of the connective tissue If, on the other hand, a virus is 'adjusted' to a non differentiated mesenchymal cell, the morbid processes will be characterised by the kinds of cells into which this stem cell can be differentiated according to its normal potentiality Hence both erythroleukæmia and myelogenous leukæmia can be produced by a virus that has affinity for a hæmocytoblast, while a virus corresponding to a still less differentiated hæmohistioblast may produce both kinds of leukæmia when the hæmohistioblast is differentiated to basophil erythroblasts and myeloblasts and endotheliomatous tumours or even spindle cell sarcomata when the same mesenchymal stem cell differentiates to endothelial cells or connective tissue cells This explanation of mixed strains is hardly compatible with the common finding with regard to malignant tumour cells—and these diseases must be regarded as malignant tumours—that their ability to differentiate is diminished or destroyed

The other theory does not define so strictly the cell specificity of the virus Although the latter may be

endothelioma to the Rous sarcoma I and other fowl tumours caused by viruses

The various strains of virus responsible must undoubtedly be regarded as being intimately related, though each has its own well defined characteristics, since it causes proliferation of particular cells in the fowl organism and of those cells only These questions will be further discussed in the next chapter, but it may be stressed here that no fowl virus which produces erythroleukæmia, for instance, has ever been seen to change so that it causes only fibrosarcomata, for example, nor has the reverse been observed The specific cell affinity of a virus is constant, and this statement is not altered by the fact that the potentialities of a leukæmic virus to produce endotheliomata in some strains have not been observed until after many passages The virus must be supposed to have possessed these potentialities from the outset but to have lacked the opportunity of manifesting them earlier

It is conceivable that mutative changes of virus may take place in the course of the passage of a strain, so that, at a given time, there are both a non mutated (original) and a mutated virus in the strain, each with its own specific cell affinities, but this possibility has never been proved

The connections between the various neoplastic conditions in the mixed strains, and the relation between the latter and the purer leukæmic strains, have been the subject of many studies and investigations There are two alternative possibilities, fundamentally different, which may offer an explanation of these features One is that the virus of a strain—whether it be regarded as exogenous or as a cell product of endogenous nature—is extremely specific as regards cells, and is capable of inducing the changes on which neoplasia depends in one kind of cell and at one definite stage of differentiation only The

It can be seen that the less differentiated the cell that characterises the strain the more cases will there be of sarcoma in addition to the leukæmic cases typical of the strain, the percentage of takes will also be higher and the course of the disease more rapid

The fact that subcutaneous injection of leukæmic blood may result in the occurrence of spindle cell sarcomata in these strains seems to support the second theory, that the virus can directly influence other cells in addition to those on which it is mainly effective, it is, however more difficult to reconcile with the first theory attributing such sarcomata to differentiation of the 'stem cell' affected by the virus which is common to both blood cells and connective tissue

Another observation tending to support the second theory is that the virus of fowl leukæmia (as well as that of some sarcomata) has been able in certain cases to produce leukæmia—or sarcoma—in pheasants, turkeys and guinea fowls (p 65) Findings of this kind seem to indicate a certain elasticity in the cell specificity of the virus and are hardly compatible with an extremely strict cell specificity

These theories will not be discussed further here, since some of the considerations are purely speculative So long as the nature of the virus, the histogenesis of the cells and the nature of the changes caused in the cells by the virus remain obscure it will hardly be possible to solve such problems

specially directed towards a certain kind of cell, for instance a hæmocytoblast, it is also able to affect, though to a lesser extent, the nearest 'relations' of this cell, both ancestors and descendants This explains why a certain type of disease—in this particular instance stem cell leukæmia—is by far the most frequent in the transmission passages of the strain, though cases of endotheliomatous tumours (in which the ancestors of the stem cells are also involved) or of myelocytic leukæmia (in which the descendants of the stem cell are caused to proliferate by the action of the virus) also occur This

TABLE II

Distribution of Leukæmia and Sarcoma in three Fowl leukæmia Strains

	Percentage of takes	Average duration of disease (days)	Percentage of		
			pure leukæmia	leukæmia + sarcomata	pure sarcomata
Strain E S (1458 chickens)	99	8·5	74·4	17·9	7·7
Strain T (994 chickens)	96	11·5	95·2	4·2	0·6
Strain R (2241 chickens)	94	15·0	98·3	1·4	0·3

theory provides an explanation why endothelial sar comata or spindle cell sarcomata can be produced in leukæmic strains occurring relatively easily in stem cell leukæmic strains less frequently in erythroleukæmic 'immature' strains, and never in relatively 'mature' erythroleukæmic strains

The above table shows the details of the three strains E S, T and R (Uhl, Engelbreth Holm and Rothe Meyer 1936*b*), of which strain E S is character ised by non differentiated hæmohistioblasts, strain T by immature hæmocytoblasts and strain R by hæmo cytoblasts differentiated towards erythroblasts

Furth and Miller filtered virus in diluted plasma through Berkefeld filters N and W and through collodion membranes They estimated that the virus was considerably less than 250 mμ in size Schaaf also was able to filter the virus with both Berkefeld filters and membrane filters having a pore diameter of 0 6 μ The percentage of takes was not so large with the filtered as with the unfiltered material and the development of the disease was slower, both features indicating a quantitative decrease in the amount of virus in the filtrates

No experiments have been published in which the size of the virus of fowl leukæmia has been established by ultracentrifugation or by filtration through Elford s graded collodion membranes, but from the experiments referred to above it must presumably be approximately the same as that of the Rous sarcoma virus which measures about 65 to 75 mμ

It has not been possible so far to demonstrate the virus of fowl leukæmia by special staining or by photography with ultraviolet light No elementary bodies have been described in material containing the virus

THE INFLUENCE OF PHYSICAL AND CHEMICAL AGENTS

Heat and Cold—The virus of fowl leukæmia is thermolabile (Ellermann and Bang, 1909, Jarmai, 1930 31, Furth 1932*c*), since it is destroyed in 30 minutes at 56° C At 37° C it is destroyed in from 2 14 days at 4° C it is active after 14 days, and it can tolerate lower temperatures Its activity is preserved for a long time by drying under suitable conditions After being dried in the frozen state as in the Flosdorf Mudd apparatus, it will keep *in vacuo* for months

Glycerine—The virus is resistant to glycerine After keeping in 50 per cent glycerine for 104 days it is still active (Furth, 1932*c*)

CHAPTER IV

VIRUS OF FOWL LEUKÆMIA

It has been mentioned that as early as 1908 Ellermann and Bang demonstrated that fowl leukæmia could be transmitted to healthy birds by means of a cell free agent obtained either by centrifugation or by Berkefeld filtration They supposed that this agent belonged to the group of ultravisible filterable viruses All subsequent research has confirmed this assumption, but not much more is known to day than in 1908 about the actual nature of the virus Many studies are available on the subject of the virus of fowl leukæmia in various conditions, on immunity against it, on attempts to cultivate it, but we still do not know what it really is No definite answer has yet been given to the fundamentally important question as to whether it consists of an exogenous living infectious substance or whether it is an endogenous cell product, the outcome of diseased cells

FILTERABILITY OF FOWL LEUKÆMIC VIRUS

Jarmai (1930 31), Furth and Miller (1932) and Schaaf (1936) investigated the filterability of the virus Jarmai noted the inconsistency that is often evident in such experiments, some filtrates being quite inactive while others, obtained with the same filter, contained the virus concerned By means of the Zsigmondy Bachmann membrane filter with a pore diameter of from 20 to 100 mμ he obtained an active filtrate When, however, an 'Ultrafilter feinst' (pore diameter less than 20 mμ) was used, the filtrate was free from albumin and the virus was removed

ing inactivation to be irreversible and so it undoubtedly is if the action lasts long enough, on the other hand as the experiments quoted above show a less energetic oxygenation may cause an almost total inactivation which is reversible

Adsorption to Erythrocytes — Engelbreth Holm, Rothe Meyer and Uhl (1933 1935) showed that the virus of fowl leukæmia can be adsorbed on normal fowl erythrocytes They mixed normal erythrocytes and leukæmic plasma and, after the mixture had stood for some time, separated the erythrocytes by centrifugation The contaminated erythrocytes were washed several times in saline and were then injected intravenously into healthy birds this resulted in about 50 per cent of takes while the plasma produced takes in about 80 or 90 per cent of the birds That this was not a specific combination of the virus with the cells was proved by experiments in which the virus was adsorbed in exactly the same way on the erythrocytes of pigeons rabbits sheep and man Attempts to extract all the virus from a plasma did not succeed in spite of many repeated adsorptions

That only half as many takes resulted from contaminated normal erythrocytes as from leukæmic plasma may be due to purely quantitative causes, but it is also possible that the virus in such cases is partially inactivated by oxygenation Ruffilli's (1938) experiments in which normal erythrocytes saturated with oxygen were mixed with leukæmic plasma and allowed to stand for 24 hours (instead of for one hour as in Rothe Meyer s experiment) before they were again isolated, supports this possibility These contaminated erythrocytes were then found to be incapable of producing one infection in six birds The fact that the six birds appeared to be immune to subsequent inoculations with leukæmic plasma suggests that an adsorption of the virus had taken place but that the latter was inactivated

Oxygenation.—The virus is destroyed or inactivated by oxygenation, and this is presumably why it is inactivated if allowed to stand; the higher the temperature, the more rapid the destruction. Destruction at temperatures over 56° C. must, however, be regarded as due to heat denaturation. The properties of the virus in relation to oxidation and reduction are very similar to those of the Rous sarcoma virus. Mueller demonstrated as early as 1928 that a filtrate of sarcomatous tissue, which normally is soon inactivated by oxygenation, can long retain its activity if cysteine or hydrocyanic acid be added (Gye and Purdy, 1930). Pirie and Holmes (1931) subjected these facts to closer examination and concluded that the virus of the Rous sarcoma was inactivated by oxidation processes, but that in suitable circumstances, virus inactivated in this way could at any rate be partially re-activated by reduction.

Corresponding experiments were carried out with the virus of fowl leukæmia by Engelbreth-Holm and Frederiksen (1938*a*), who found that oxygenation—by simply bubbling oxygen through leukæmic plasma at 18° C.—inactivated the virus in the course of from 15 to 30 minutes, the injection of the plasma into healthy chickens being then no longer able to produce takes. Attempts to re-activate such oxidised virus by reducing it in a cysteine-cobalt sulphate system, as used by Pirie and Holmes, succeeded in two experiments out of five. In these experiments a plasma which before treatment produced takes in 17 out of 19 birds after oxygenation for 25 to 35 minutes produced only 4 takes in 18 birds. After reduction this inactivated plasma again produced takes in 16 out of 19 birds.

Ruffilli (1938 *b*, *g*) inactivated the virus of fowl leukæmia both by bubbling oxygen through the leukæmic plasma and by treatment with hydrogen peroxide and permanganate of potassium. He considered the result-

Attempts have also been made to influence the virus of fowl leukæmia by the action of thorium X (Wallbach, 1932, Jarmai, 1932), though without convincing results

In this connection it should be mentioned that various attempts to cure fowl leukæmia by medicaments which were intended to 'kill' the virus have been recorded Jarmai (1932) attempted treatment with Atoxyl and benzene, but produced no cures Schaaf (1936) tried Optarson and Solarson (arsenical preparations) and other chemotherapeutic substances without success Zadik (1933) thought that he had cured 10 out of 20 birds suffering from erythroleukæmia by treatment with a lead preparation known as R 237 b (Rothmann) Oberling Guerin and Guerin (1934) thought similarly that they had obtained therapeutic effects with Plasmoquine and Rhodoquine

Engelbreth Holm Rothe Meyer and Uhl repeated these experiments in 1935 with a large number of birds with the same technique, and with mainly the same strain of birds (Engelbreth Holm s strain R) The results indicated that the lead preparation R 237 b had no effect on fowl leukæmia but that it had a violent toxic action on fowls Plasmoquine was also quite ineffective These results have been confirmed by Schaaf (1936) Rhodoquine seemed to have a slight effect both *in vitro* and *in vivo* the course of the disease being somewhat prolonged and the virus apparently weakened to some extent *in vitro* in the case of one strain (R) though no effect was observed in another (E S)

It has already been mentioned that Engelbreth Holm (1933) investigated the action of liver preparations (among others Campolon) on erythroleukæmia but obtained no result Schaaf (1936) also tried liver treatment but it proved to be ineffective The same authors tried to influence the course of the disease with various hormones (gonadotropin thyroidin folliculin growth hormone) but without success

ISOLATION OF VIRUS

Only a few attempts to isolate the virus of fowl leukæmia have been recorded Jarmai (1938) separated virus containing material (hydrolysed leukæmic blood

Irradiation — The virus of fowl leukæmia is resistant to irradiation Jarmai (1930 31) irradiated virus containing material with ultraviolet light at a distance of 15 cm for one hour, without being able to demonstrate any decrease in the infecting power, few technical details of this experiment are given, however, and it would seem possible that, owing to the thickness of the layer irradiated, some of the virus may have escaped irradiation

The virus is extremely resistant to X rays Though Jarmai (1932), after an exposure of leukæmic fowls to X rays, found a decrease in the number of patho logical cells in the blood, cures were not obtained He thought, however, that he had observed destruction of the virus in the organism in one case after radiation with X rays (63 KW, 6 mA, distance 34 cm , no filter, five 20 minute treatments in 9 days), since attempts at transmission to three birds produced no take Engel breth Holm and Rothe Meyer (1932*a*) observed a similar result from universal irradiation for 53 minutes of a leukæmic fowl, whose blood was previously able to produce takes (180 KW, 4 mA, distance 33 cm , filter 1/2 mm Cu+3 mm Al) Subsequent attempts at transmission to three birds gave negative results The result of another similar experiment was, however, that the ability of the blood to produce takes was the same after irradiation as before (Rothe Meyer and Engelbreth Holm, 1933*a*) No decrease in the power of the virus to take was produced by irradiation of blood *in vitro* with up to 5500 r Jarmai (1938 39) repeated these experiments and found that on irradiation *in vitro* the virus could tolerate doses of even about 130,000 r Forfota (1937*a*, *b*) obtained similar results

These investigations show how much care must be taken in assessing such studies Only results that can be reproduced in several experiments involving a fair number of birds can be considered reliable

cultivating the virus for several passages when the type or types of cells against which the virus was directed were growing in the culture If this were not so, no survival and certainly no multiplication of virus occurred Fibroblast cultures of the bone marrow of fowls with pure erythroleukæmia, for instance, did not contain virus The combined leukæmic sarcomatous strains of virus could be cultivated in cultures of the sarcoma concerned, while the virus of 'pure' leukæmic strains could be cultivated *in vitro* only if the cultivation of the blood cells concerned was successful This occurred with the myeloblasts in strain 1, which survived and grew in cultures in which virus was also detectable for periods up to 30 days Furth concluded from his comprehensive experiments that these viruses multiply *in vitro* only in the presence of cells on which they confer neoplastic properties" These experiments, in which virus from mixed strains can still produce both leukæmia and sarcoma after cultivation *in vitro* in sarcomatous tissue prove the justice of the assumption that the different manifestations of the disease with these strains are due to one virus only which can stimulate both primitive blood cells and kindred mesenchymal cells

Ruffilli (1937) reported in apparent contradiction of these findings that cultures of fibroblasts from the myocardium of leukæmic fowls, cultivated in the usual manner in Carrel flasks and by the cover glass method, can produce typical erythroleukæmia on inoculation after 122 days (44 passages) A possible explanation of this may be that the strain was not purely erythroleukæmic but mixed, an assumption which is perhaps supported by Ruffilli's statement that the fibroblasts in the cultures were somewhat atypical and resembled neoplastic cells

Ruffilli's (1938*f*) report of experiments in which cultures of normal fibroblasts from the hearts of chickens

corpuscles) into one fraction of albumin and one of globulin, by precipitation with ammonium sulphate After these fractions had been washed and dissolved, the greater part of the virus was found in the globulin fraction, with which the percentage of takes was 100, while it was only 20 with the albumin fraction Morelli and Vercellone (1938) precipitated leukæmic plasma with ascorbic acid (1 150) The resulting precipitate was incapable of transmitting the disease, while the liquid remaining produced transmissions in healthy fowls

CULTIVATION OF VIRUS

This has been attempted by several investigators The virus can be cultivated outside the organism only in intimate connection with living cells

Verne, Oberling and Guerin (1936) attempted to grow the virus in tissue cultures of the bone marrow of fowls After a few passages such cultures consisted of fibroblasts, after which the virus could no longer be demonstrated Cultivation in Carrel flasks with repeated additions of fresh marrow from normal hens produced successful takes after 15 days, but the authors think that this was merely a question of survival of the virus and that there are no grounds for the assumption that the virus had undergone multiplication

Furth and Stubbs (1934) also found that the blood cells disappeared from tissue cultures in the course of a few days They succeeded, however, in cultivating sarcomatous tissue from one of their mixed strains (strain 13, erythroleukæmia combined with endothelial sarcoma) After 67 days fowls were inoculated with these cultures, which then appeared to be extremely 'virulent producing both sarcoma and erythroleukæmia —as was usual with this strain—in 5 out of 6 birds Similar experiments with more strains (Furth and Breedis, 1937) demonstrated the possibility of preserving and



Fig 36 —Chorio allantoic membrane of a fertilised hen's egg after incubation for 40 hours Some primitive cells of the basophil erythroblastic or hæmocytoblastic type are seen × about 500

were 'infected' with the leucocytic layer from erythro leukæmic blood was still more remarkable The fibro blasts were subsequently subjected to further cultivation, which caused the blood cells to disappear from the fourth passage, whilst the fibroblast cultures were able to produce takes in two birds up to the ninth passage (22 days) Whether this was a case of the continued co existence of blood cells, in spite of the fact that they were not observed, or whether the case was one of real *infection* with fibroblasts as in other fowl tumours (Ludford, 1937) is still uncertain

Pending confirmation by other workers of Ruffilli's results it seems wisest to assume that the virus of fowl leukæmia, like that of fowl sarcomata, can be cultivated *in vitro* only in the presence of cells against which it is directed and which it can influence with the resulting development of leukæmia—or possibly sarcoma *

Finally, attempts have been made to cultivate the virus of fowl leukæmia in fertilised hen's eggs, using the technique of Woodruff and Goodpasture (1931) Jarmai (1933*a*) 'infected' fertilised hen's eggs by inject ing virus containing substance (blood) directly into the egg, and found that the embryo could not be infected until it was ten days old The development of the bone marrow in fowl embryos does not begin until the ninth day, and Jarmai therefore concluded that the presence of bone marrow cells must be a condition for the cultiva tion of virus by this means Storti and Mezzadra (1938) also employed Woodruff and Goodpasture's (1931) technique, and found that the virus of fowl leuk æmia could live in the fertilised hen's egg even before

* Since this book was written however Ruffilli s work appears to have been confirmed by Doljanski and Pikovski (1940) who found that cultures of heart muscle from the leukæmic fowl were infective after six months and who also claim to have cultivated the leukotic agent by infecting cultures of normal fibroblasts with infective filtrates

the tenth day of development of the embryo, they did not, however, provide any proof of the multiplication of virus, since living virus was not demonstrated in the chorio allantoic membrane for more than three days (3 cases) and four days in one case No serial cultivation of virus was made These authors were of opinion ('bien que pas encore complètement sur') that most probably multiplication of the virus had taken place, *i e* multiplication of the virus without the presence of cells specifically sensitive to the virus This, however, has not been demonstrated with certainty Even successful cultivation in passage of the virus of fowl leukæmia on the chorio allantoic membrane, which has not yet been achieved, will not necessarily indicate that the virus can grow if it is not connected with the cells that are specifically sensitive to it, for the chorio allantoic membrane of fowl embryos contains cells of this kind, *i e* primitive endothelial cells and stem cells, from the second day of development (Fig 36)

The assumption that the virus of fowl leukæmia can be cultivated only in association with cells specifically sensitive to it is, in fact, affected just as little by the results of attempts at cultivation in the egg as by the experiments with tissue cultures

CONDITIONS OF IMMUNITY

Everyone who has been engaged to any large extent in transmission experiments with fowl leukæmia has come across a few birds which do not react to the inoculation of leukæmic material by developing leukæmia, but seem to be resistant Moreover, isolated cases of spontaneous recovery from the disease have occasionally been observed Birds which have once withstood inoculated leukæmia often appear to be immune to the virus in subsequent inoculations, though this immunity is not absolute and does not seem to be permanent These

by development of the disease may quite well be an acquired immunity developing so rapidly and to such an extent that the pathological symptoms do not evolve to a point at which they can be recognised Serial experiments in which the content of virus in the blood after inoculation has been observed for long periods seem to indicate clearly the correctness of this assumption A similar opinion as regards resistance and immunity to transplanted tumours was advanced by Woglom (1929)

It is interesting that such immune fowls are generally also immune to strains of virus other than that which caused the immunity This feature, too, reflects observations on fowl sarcomata caused by viruses (Andrewes, 1931, 1932) Oberling and Guerin (1937) further demonstrated that fowls which are immune to the inoculation of leukæmia are also immune to transplantation of sarcomata produced by the same virus

As already mentioned, the plasma of these fowls contains virus neutralising substances which can withstand heating to 56° C for 45 minutes but which cannot inactivate cell containing material Rothe Meyer, Engelbreth Holm and Uhl (1935) also demonstrated a virus neutralising property in the plasma from a fowl with slowly progressive erythroblastic anæmia after the destruction of the virus in the plasma by heating to 56° C This further supports the theory that such anæmias are to be regarded as erythroleukæmia in an attenuated form The observation of these authors that serum from a spontaneously recovered fowl which was shown to contain virus neutralising substances was unable, on later examination, to inactivate the virus serves to illustrate the fact that acquired immunity is not always permanent Such experiments must however, be taken with a certain amount of reserve, since

phenomena have not yet been fully explained A distinction should presumably be made between immunity to virus and immunity to cells (Furth, 1932*a*), as in the case of other fowl tumours caused by a virus, but this point also awaits further elucidation

Furth (1932*a*) demonstrated that serum from 'immune' fowls could to a certain extent inhibit the power of a leukæmic serum to infect *On the other hand, he* did not succeed in inhibiting cell containing infective material

Rothe Meyer and Engelbreth Holm (1933) investigated *similar conditions and obtained similar results*, namely that the plasma of birds which have recovered spontaneously can neutralise virus in leukæmic plasma but not in cells Normal plasma does not possess this property It is also emphasised that the immunity derived from spontaneous recovery is not always permanent For instance a case has already been mentioned *of a fowl which survived nine re inoculations* after spontaneous recovery but died from leukæmia after the tenth

Rothe Meyer (1934) investigated the incidence of the so called resistance in fowls and found that this was never observed in chickens under eight weeks old Only three resistant birds were found among 1900 chickens *over two months old No definite figures can be given* for adult fowls since the resistance as already mentioned varies with the season, on an average about 2 per cent of fowls are resistant *As regards the* frequency of spontaneous recovery after inoculation, Rothe Meyer found 26 recoveries out of 1060 adult fowls which had shown leukæmic blood changes *He* emphasised that it is perhaps wrong to make a distinction between natural resistance and spontaneous recovery in this way since a resistance that is indicated by the fact that inoculation of leukæmic material is not followed

even the infective potency of cell containing material Takes were produced in only twenty four of eighty six birds (about 28 per cent) in five different experiments in which erythroleukæmic blood and immune duck serum were mixed in the ratio 1 20 or 1 200 and, after standing for some time, were injected into the chickens The same quantity of the same blood, after standing for the same time without duck serum, infected forty seven out of fifty three birds (about 89 per cent) That the duck serum had a pronounced inhibiting effect on the virus in the blood was further indicated by the fact that the twenty four chickens which developed leukæmia after injection of the mixture of blood and immune serum did not die until, on an average, twenty days later, while the average life of the forty seven control birds was only nine days

The union between virus and antivirus was found to be fairly stable Thus, virus could not be liberated by dilution with saline, as can the combination vaccine virus/antivirus, nor could the combination Rous sarcoma virus/antivirus be liberated in this way (Andrewes, 1932) Uhl further investigated the question whether the neutralisation of leukæmic virus (from several strains), and the inhibition of virus in cell containing material which is produced by serum from ducks immunised with the blood of leukæmic fowls, are due to antibodies directed against fowl protein as such, or whether the presence of an antivirus must be taken into consideration Eleven ducks were accordingly immunised with normal adult fowl blood, seven with normal chicken blood and three with embryonic fowl tissue Serum from these ducks and leukæmic plasma were mixed exactly as in the previous experiments and injected intravenously into chickens In seven different experiments takes resulted in fifty six out of sixty one birds (about 92 per cent), while sixty one out of

in practice it is not feasible to make a quantitative estimate of the virus content of leukæmic plasma until the latter is tested for inhibitory properties A definite amount of immune serum can undoubtedly neutralise only a certain limited amount of virus

No details are available of the nature of the reaction that takes place between the virus and the virus neutralising antibody in fowl leukæmia The usual serological reactions—precipitation and complement fixation—when they occur, do not run parallel with virus neutralisation Thomsen, Engelbreth Holm and Rothe Meyer (1933) found no complement fixing antibodies specific to virus, but only those produced by the injection of foreign blood into the blood of an erythroleukæmic fowl

ACTIVE IMMUNISATION AGAINST FOWL LEUKÆMIA VIRUS

Ducks —Uhl, Engelbreth Holm and Rothe Meyer (1936*a*) demonstrated that it was possible to immunise ducks actively to the virus of fowl leukæmia Repeated (ten) injections of leukæmic fowl blood every other day produced antibodies to virus, as well as to fowl blood itself, in the sera of these birds Normal duck serum was found to lack the virus neutralising effect Uhl (1937) thoroughly investigated this phenomenon He immunised more than sixty ducks in the manner described and in twelve different experiments found a pronounced virus neutralising effect exerted by the sera of these birds The sera of the ducks neutralised not only the virus with which they had been immunised (Engelbreth Holm and Rothe Meyer's strain E S) but also that of another leukæmic strain (strain R) In addition to neutralising virus in serum or plasma (in several experiments 1 c c of duck serum completely neutralised the virus in 1 c c of leukæmic plasma), the immune serum from ducks was able to some extent to weaken

of virus antibodies to the fowl antigen antibody complex has not been decided It is a well known fact that the antigen antibody complex possesses a great power of adsorption

These duck sera, which had shown such a pronounced neutralising effect on free virus, and could to a certain extent inhibit virus combined with cells, were used in a series of attempts to inhibit the development of the disease in chickens inoculated with virus (Engelbreth Holm, Rothe Meyer and Uhl, 1936) Two series of experiments were carried out in which chickens inoculated with leukæmic plasma were treated with 10 c cm of duck serum immediately after inoculation (110 birds) or with 10 c cm on the third day and 4 c cm on each of the two following days (thirty seven birds) No change whatever in the course of the disease resulted in one series of experiments, since eighteen out of twenty birds died from leukæmia just as rapidly as the control birds Only fifteen out of twenty seven birds treated in the other experimental series developed leukæmia, against twenty three out of twenty six control birds In this series, therefore the treatment may have been to some extent effective

Fowls — Active immunisation of fowls with leukæmic virus has been attempted many times In 1920 Ellermann and later Engelbreth Holm (1932), Jarmai (1932) and Schaaf (1936) attempted to produce immunisation by the subcutaneous and intramuscular injection of various virus containing substances The desired result was not obtained either by injections of small and increasing doses or by treatment with virus attenuated in various ways

Oberling and Guerin (1938*b*) attempted to immunise fowls by the intracutaneous injection of small quantities of blood This method also did not produce any certain immunity Rothe Meyer Engelbreth Holm and Uhl

sixty three control birds developed leukæmia (about 97 per cent) The birds in the first group died on an average after fifteen days, whilst the control birds lived for about eleven days How this slight variation in the two groups should be interpreted is doubtful It should be mentioned that the difference is largely due to the result of one of the seven experiments, in which only six out of ten inoculated birds developed leukæmia, whilst in the other six experiments takes were observed in fifty out of fifty one birds, i e the same result as that in the control group (sixty one out of sixty two) Uhl is certainly right in his conclusion that antibodies to fowl protein cannot explain the inhibiting effect of the duck sera on cell containing material, and still less their often total neutralisation of cell free virus containing plasma This is clearly illustrated by an experiment in which some of the ducks which had been immunised with normal fowl blood with no development of virus neutralising antibodies were subsequently immunised with leukæmic blood, after which their serum completely inactivated leukæmic plasma

Nor can the assumption that such neutralisation requires the presence of complement explain the defective virus neutralisation of duck serum after immunisation with normal fowl blood since the addition of fresh guinea pig serum did not affect the results of these experiments

Uhl also found evidence for the assumption of a specific antivirus in the duck serum by absorbing it with normal fowl blood corpuscles until it was no longer able to agglutinate them, it still retained its virus neutralising property Titration however showed that absorption with normal fowl blood corpuscles to some extent reduces the virus neutralising property Whether this is a matter of non specific mechanical adsorption

chickens died of leukæmia, as against four out of five control birds The eight surviving fowls and seven chickens were now given three injections of increasing doses of leukæmic blood, the last being 1 c cm These injections which produced twenty nine takes in thirty control birds resulted in the development of leukæmia in four fowls and four chickens, whilst four fowls and three chickens still appeared to be immune This result agrees with those obtained when the same method is used for the immunisation of mice and chickens against fowl plague—30 50 per cent of the animals so treated become immune That four of ten fowls and three of eleven chickens had really been immunised was indicated by the fact that Uhl afterwards demonstrated the presence of virus neutralising antibodies in their serum Some immunity had presumably also developed in the birds that died from leukæmia during the experiment since the course of the disease was longer (average eighteen days) than in the controls (average eleven days)

Thus in fowl leukæmia it has now proved possible to effect active immunisation, in spite of the earlier negative experiments

It will be evident from the investigations reported in this chapter that as regards immunisation the various known strains of fowl leukæmia virus—both pure and mixed—show a remarkable resemblance to the char acteristics observed in the case of fowl sarcoma viruses Though the latter have generally been subjected to far more thorough study * the similarity on almost every point is so surprising that one is bound to assume that the two types of virus are intimately related an assumption which was almost inevitable when the combined sarcoma leukæmic strains became known

* For details of the investigations into the Rous and other fowl sarco mata reference should be made to O Thomsen in Doerr and Hallauer s *Handbuch der Virusforschung* Vol II 1939 p 994

(1935) tried to induce active immunity with mixtures of virus and antivirus, with negative results Jarmai (1935*b*) attempted to immunise fowls with extracts of fowl embryos, but this had no effect on the results of subsequent inoculation with virus The same author (1932) had previously tried to produce immunisation with virus neutralising sera

It has already been mentioned that the virus of fowl leukæmia is inactivated by oxygenation Ruffilli (1938*b*) inoculated ten birds with such inactivated virus Four were given two injections of oxygenated leukæmic plasma at an interval of twenty days Two of these survived a subsequent injection of extract of leukæmic spleen Six birds which had been immunised with leukæmic plasma inactivated by means of normal red blood corpuscles saturated with oxygen, were immune to a subsequent inoculation of leukæmic plasma From this Ruffilli concluded that virus which has been in activated by oxygenation retains its antigenic property

Uhl (1938) obtained real active immunisation of chickens and fowls by the injection of leukæmic virus adsorbed to aluminium hydroxide (Wilstätter type C), he used a technique described by Schmidt, Ørskov and Hansen (for literature see Uhl, 1938) for immunisation against different toxins and ultramicroscopic viruses

Uhl adsorbed on aluminium hydroxide the virus in extracts of spleen and blood from erythroleukæmic fowls After standing for some time the aluminium hydroxide was removed by centrifuging and kept at a temperature of $-5°$ C Ten fowls and eleven chickens were then treated by subcutaneous injections of increas ing quantities of this aluminium hydroxide twelve injections in all being given at intervals of four days One month after the last injection 1 0 c cm of leukæmic plasma was injected intravenously into each of the birds Two of the ten fowls and four of the eleven

TRANSMISSION EXPERIMENTS IN CATTLE

Leukæmia has not been successfully transmitted in cattle, although slight transitory changes in the blood, similar to those in Ludke's dog experiment, have been observed in a few cases

Knuth and Volkmann (1916) inoculated three calves with minced lymph node tissue from a leukæmic cow and fed six dogs with liver tissue from the same animal In addition, the blood of the cow was injected intravenously into the calves Four calves together with guinea pigs and mice, were used in similar experiments The blood of the calves was subsequently examined some of them had reacted with the development of lymphocytosis but no leukæmias were observed

du Toit (1916), Endres (1921) Creech and Bunyea (1929) Schottler and Schottler (1934), Dobberstein and Piening (1935) and Stasney, Feldman and Popp (1939) were equally unsuccessful in obtaining transmission in similar experiments with cattle Schottler and Schottler (1934) however stated that, after inoculating the animals with *leukæmic blood intravenously or with leukæmic* lymph node tissue subcutaneously they found transitory lymphocytosis, amounting to 80 90 per cent of the total leucocytes the actual number of which was not however, given Normally, a cow has about 10,000 leucocytes per c mm of which about 50 per cent are lymphocytes and a calf has about 12,000 to 15,000 per c mm , of which about 80 per cent are lymphocytes (du Toit 1916) A further transitory lymphocytosis, which never developed into leukæmia, was regarded by Schottler and Schottler as a latent stage of lymphogenous leukæmia, but this opinion would seem to lack foundation

Dobberstein and Piening (1935) observed similar lymphocytosis after inoculation with leukæmic blood or minced lymph nodes In some cases they also found

CHAPTER V

TRANSMISSION OF MAMMALIAN LEUKÆMIA

TRANSMISSION EXPERIMENTS IN DOGS

It has already been mentioned that the first attempts to transmit leukæmia from one mammal to another of the same species were made by Bollinger, who, in 1874, tried to transmit lymphogenous leukæmia from one dog to another by injecting an emulsion of splenic tissue into the tissue of the lungs. Cadiot (1892) made similar experiments with several kinds of animals; he gave subcutaneous, intraperitoneal and intravenous injections of cell containing material from the organs of a dog with lymphogenous leukæmia to twelve dogs, three cats, two goats, two rabbits and twelve guinea pigs, but no take resulted. Émile Weil and Clerc (1904*b*) also attempted transmission in dogs by intravenous and intraperitoneal injections of blood and by subcutaneous transplantation of lymph node tissue, but did not produce the disease. Dahlstrom and Henschen (1918), and Wirth (1920) were no more successful in their attempts to transmit dog leukæmia to dogs. A doubtful result was obtained by Ludke (1910) from the intravenous injection of emulsions of bone marrow and spleen from a leukæmic dog into six young dogs. One of these shortly afterwards displayed a surprising blood picture with 100 000 leucocytes per c mm., including a few myelocytes. The hæmoglobin percentage also fell slightly and a few normoblasts were observed. There was, however, no manifest development of leukæmia, and the changes disappeared spontaneously.

month four of the five calves displayed moderate anæmia and the number of white blood corpuscles rose, without, however, reaching extraordinarily high values. The increase consisted largely of lymphocytes of which a very few were immature, large, with leptochromatic nuclei and a few nucleoli. All these changes receded spontaneously.

How these transitory changes are to be interpreted is doubtful. It is obvious that they cannot be regarded as successful transmissions. The possibilities to be considered are either that this is just a non specific reaction or that there is a transplantation which retrogresses rapidly as is often seen when animals genetically different from the donor are inoculated with tumour substance. The appearance of immature lymphocytic cells in the circulation may perhaps point in this direction. Further research is necessary, however, before these possibilities can be profitably discussed.

TRANSMISSION EXPERIMENTS IN RODENTS

Although as indicated above the transmission of leukæmia to healthy animals has not been attained in the case of the larger mammals transmissible leukæmia and kindred sarcomata are well known in rodents.

Schultze (1914) reported experiments in which he had transmitted a lymphosarcoma in rabbits for eighteen months by the subcutaneous or intramuscular implantation of tissue. Very widespread changes closely resembling lymphogenous leukæmia were observed in many of the inoculated animals. Blood changes were also seen in two cases (*e g* 45 000 leucocytes per c mm with 21 per cent lymphocytes and 70 per cent 'sarcoma cells')

The pictures resemble those of Sternberg's leucosarcomatosis and Schultze emphasised the close connection existing between lymphosarcoma sarcomatosis

moderate anæmia (up to 20 per cent decrease in the total number of erythrocytes) and temporary fever This state was regarded as the initial stage of lymphogenous leukæmia in spite of the fact that manifest leukæmia was *not observed in any case* From these experiments which included two control cows injected with normal serum without showing similar changes, the authors concluded that cattle leukæmia could be transmitted by means of a virus which they claim to have investigated in various ways The experiments reported do not, however, justify such a conclusion In *no case has transmission of the disease been accomplished* and serum from the affected animals—often with slight lymphocytosis, etc —behaves like normal serum when transmitted further du Toit (1916) reported that the injection of minced organs of normal animals also produced leucocytosis in cows

Stasney, Feldman and Popp (1939) injected *lymphogenous leukæmic blood intravenously and minced lymph* nodes subcutaneously into two calves, one of which had previously been irradiated over the splenic region with X rays The blood of both animals was examined before and after inoculation No anæmia developed but there was a considerable leucocytosis (81,000 and *88 000 leucocytes per c mm) with many myelogenous* cells, some of them immature A few lymphocytes with immature nuclei were found in both animals, reminiscent of those seen in the donor beast The changes reverted to normal after ten days, and thorough examination (*including autopsy) 283 and 346 days* later revealed no sign of leukæmia in either animal

Similar results were obtained by Engelbreth Holm and Plum (unpublished) These workers injected the blood of a leukæmic bull (lymphogenous subleukæmic leukæmia) into five young calves *one of which was the* offspring of the leukæmic donor During the following

abdominal tumour composed of pathological myeloblasts, which were also observed in the blood to an amount of 6 per cent No leukæmic changes were found in the organs The tumour proved to be transplantable when an emulsion of it was injected intraperitoneally Single or multiple neoplastic nodes composed of the same cells developed on transmission by this means, but no true leukæmic picture was observed

Oberling, Guerin and Guerin (1939) as already mentioned, described several carefully investigated cases of leukæmia, both lymphogenous and myelogenous in rats From five out of six animals with lymphogenous leukæmia these authors tried to transmit the disease to groups of ten to thirty rats but no definite transmissions were obtained Seventeen months after inoculation a case of lymphogenous leukæmia was found in one group and after nine months two cases of sarcoma of the intra abdominal lymph nodes were found in another Leukæmic blood changes were present in one of these cases but Oberling Guerin and Guerin were not convinced that these various developments could be regarded as the result of the inoculations they had made The remaining three groups of animals showed no evidence of transmission On the other hand undoubted transmissions were obtained with material from one of three animals with myelogenous leukæmia Twelve rats were inoculated subcutaneously with pieces of organs and pieces of coagulated blood were injected into eight Eleven months later a small myeloma was found in one of these animals at the site of inoculation and well marked myelogenous leukæmic changes were also found Twenty young rats were inoculated with fragments of liver and spleen from this animal, and ten with tissue from the subcutaneous myeloma One of the twenty developed an abdominal tumour which was regarded as a myelosarcoma Three small subcutaneous

and leukæmia, which seem to be various expressions of the same disease It will be noted, however, that Schultze observed only transplantation cases, *i e* cases in which the cells that produce the pathological processes are foreign to the organism, and which cannot therefore be compared with cases of spontaneous origin

In guinea pigs Miguez (1918) was the first success fully to transplant a lymphosarcomatous tumour this was retransmitted by Fischer and Kantor (1919) through numerous passages As in Schultze's case, there is no question here of the transmission of true leukæmia

Fraser (1925) similarly transmitted lymphosarco mata in the South American red squirrel The experi ments are not described in detail, but it is said that cases of spontaneous regression were observed among the animals inoculated Terminal blood changes are mentioned, but whether any of the cases were typical leukæmias cannot be decided

The first successful transmission of typical leukæmia in mammals was performed by Snijders (1926), who found cases of lymphogenous leukæmia in guinea pigs in the Dutch Indies It was possible to transmit the disease to healthy guinea pigs by means of cell contain ing material, and Tio Tjwan Gie (1927) submitted the matter to close investigation The disease could not be transmitted with cell free material Transitional forms between lymphogenous leukæmia and lympho sarcoma also appeared in these experiments

Transmissible leukæmic disease has also been described in rats—though not until recently It has been mentioned previously that Wilens and Sproul (1936) observed a number of cases of leukæmia in rats Trans mission was attempted from one of these, but without success

In 1938 Rask Nielsen described a rat with a large

290,000 'tumour cells' per c mm, did not develop until late in the animals' lives The picture was comparable with that of Sternberg's leucosarcomatosis in human beings

LOWERING OF RESISTANCE BY IRRADIATION WITH X RAYS

The transmission of mouse leukæmia to other mice, however, seldom succeeds if the mice used in the experiments are of mixed (miscellaneous) origin or of strains * different from that in which the leukæmia originated

This difficulty however, can be overcome, as appears from the studies of Krebs, Rask Nielsen and Wagner (1930), who found a means of lowering the resistance of mice to transplanted tumours It was shown that the resistance decreased if the mice had previously been totally irradiated with a sublethal dose (300 400 r) of X rays, the tumours in question thereafter being transmissible to a large number, and not infrequently to all, of the animals inoculated Further, the same authors made the significant discovery that the incidence of spontaneous tumours and of leukæmia increased greatly in animals irradiated in this way The interesting problems raised by the latter finding will be described in detail in Chapter IX

By the method of irradiation described above Krebs, Rask Nielsen and Wagner succeeded in establishing a transmission line of lymphogenous leukæmia with transitional forms to lymphosarcoma ('lymphomatosis infiltrans leukæmica et aleukæmica') This line has been used by Krebs and his co workers, Rask Nielsen Kaalund Jorgensen, Clemmesen, and Bichel, for a great number of experiments carried out since

* The word strain here means a series of generations of animals inbred by brother sister mating Mice of more miscellaneous breed or of mixed origin will be referred to as stock Line indicates a transplantation series of tissue inoculations for several animal passages

tumours at the site of inoculation, together with leukæmic blood changes were found in three of the ten

TRANSMISSION EXPERIMENTS IN NON-INBRED MICE

The difficulty of obtaining evidence of the experimental transmission of leukæmia in rats, rabbits, dogs and cattle does not necessarily imply that the disease is not, or is only rarely transmissible in these animals It was not until experiments were carried out with mouse leukæmia which, likewise could not be transmitted to healthy animals at the first attempts, that the extraordinary importance of the genetic factor became clear These diseases—as is the case with most transmissible tumours—can generally be transmitted only to animals which are closely related to, or belong to, the same strain the genetic constitution of which is thus similar to that of the animals from which the leukæmic or neoplastic tissue originates

As already indicated, the first attempts to transmit mouse leukæmia were unsuccessful Tyzzer (1907-08) and Haaland (1911) tried to transplant mouse lymphosarcomata and lymphogenous leukæmia into large numbers of animals, without result Levaditi (1914) also tried to transmit two typical examples of mouse leukæmia to a large number of healthy animals but without success

It was not until 1929 that Korteweg succeeded in transmitting a lymphosarcoma which had arisen in the thorax of a mouse following an injection of tar emulsion into the trachea eleven months previously The tumour was re-transmitted by subcutaneous transplantation through twenty passages lymphosarcoma as well as systematised widespread lymphogenous leukæmic changes developing in the liver kidneys pancreas spleen lymph nodes and bone marrow of the animals inoculated Leukæmic blood changes, with up to

gations Rask Nielsen furthermore expressed the view that the 'lymphomatosis' in the line under discussion should more correctly be regarded as belonging to the group of myelogenous leukæmia, since the cells characteristic of the disease show a transition to peroxidase positive, azurophile granulated cells resembling promyelocytes and since the nuclear structure is not typically lymphocytic This opinion was supported by Kaalund Jörgensen (1936) who considered that the cells were myelogenous and closely related to reticular cells Thus the history of this line provides a good illustration of the difficulties that may be involved in classifying leukæmias characterised by immature cells—difficulties which apply equally in animals and in man

The disease can be transmitted to rats after intensive irradiation (625 r to the whole animal) and can then be re transmitted to mice This kind of heterologous transplantation after the resistance of the new host has been greatly reduced, was reported by Krebs and Thrane (1932) and by Furth, Seibold and Rathbone (1933) The experiments show how very powerful is the action of X ray irradiation in lowering resistance According to Clemmesen s (1938) extensive studies (in which, however he employed tumours of other types than leukæmia) this action is due to a retardation of the immunisation processes which normally take place in an inoculated animal as a result of the introduction of antigenically foreign tissue Thus immunisation to foreign tissue can be inhibited or so greatly delayed by irradiation with X rays that the tumour tissue inoculated gains time so to speak, for growth and spread before the body s natural defence mechanism becomes effective

Finally Bichel (1939) cultivated the pathological cell of this line *in vitro* by allowing it to grow in fibroblast cultures in which its growth was of an infiltrative type, like that of tumour cells on the other hand pure

1930, they have included a series of investigations into the biological effects of X rays, which do not fall within the scope of the present work, as well as studies of the behaviour of the leukæmic tissue itself in transplantation series It emerged, *inter alia*, that after the leukæmia had been repeatedly transmitted for some years to irradiated mice, it could then be transplanted to non irradiated animals (Kaalund Jorgensen, 1936) This finding corresponds with the known behaviour of various tumours on transplantation If the first series of transplantations is successful, in spite of a low percentage of takes (as, for instance, in the experiments of Krebs and his co workers with X ray irradiation), the percentage of takes will often increase in later passages in mice of the same strain Similar conditions are observed in the other known lines of leukæmia in mice (p 109) Moreover, the use of X ray irradiation to lower resistance is applicable also to cases in which it is desired to transmit leukæmia from one strain to another, as was done by Furth, Rathbone and Seibold (1932 33)

Rask Nielsen (1936) studied the blood and tissue changes occurring in the line mentioned above, after inoculation of irradiated mice, and he followed the effects produced by the inoculated cells as they gradually spread through the organism That the leukæmic changes in various organs are produced by dispersion of the inoculated cells had been established earlier by Richter and MacDowell (1930) and Potter and Richter (1933) Rask Nielsen however, thought he had reason to believe that the changes in the bone marrow, at any rate were also partially due to a proliferation of the animal s own cells ' perhaps influenced by an agent from the leucosis cells This opinion, which was put forward with all possible reserve cannot be evaluated with any certainty by purely morphological investi

The first strain of this sort to be of particular interest to investigators of leukæmia was described by Richter and MacDowell (1929, 1930 *a*, *b*) A very large number of cases of leukæmia gradually appeared in a strain (C 58) which had been inbred since 1921 by brother sister matings In this way 90 per cent of the animals of the eighteenth to the twenty third generations, that were over six months old, died of leukæmia Aleukæmic, subleukæmic, and pronounced leukæmic cases were observed, although it was impossible to draw a line between the groups, since every conceivable transition occurred Cases were also seen in which the course of the disease changed from subleukæmic to leukæmic, and *vice versa*, *e g* from 354,000 to 31,900 leucocytes per c mm, and even from 229,000 to 3800 No cases of spontaneous recovery were seen It was as difficult to distinguish definitely between pure leukæmia and lymphosarcoma as it was between leukæmic and non leukæmic cases Of 543 cases of leukæmia 450 were lymphogenous and 6 myelogenous, while the type of 87 could not be ascertained with certainty Subcutaneous or intraperitoneal inoculation of young animals (one or two months old) with a saline emulsion of the splenic tissue from 10 animals of the same strain was followed in every case by the development of general leukæmic lesions Considerable leukæmic blood changes were found in many of these animals, with up to 260,000 white blood corpuscles per c mm, mostly immature lymphocytic cells

Transmission to mice of other strains was not successful, but four different lines originating from four different cases of spontaneous lymphogenous leukæmia were transmitted for a large number of passages by repeated transplantation of cell containing material

It generally happens in such transmission lines that the virulence increases evidence of this is to be

cultures of the leukæmic cells, which Bichel, too, regarded as myeloblastic, could not be obtained

These experiments, which were all made with the same line, illustrate in different ways the properties of the leukæmic tissue concerned The combined results indicate that this tissue must be regarded as neoplastic, since its mode of growth in the body of the host is infiltrative its immunity relationships and its behaviour in animals irradiated with X rays closely resemble those of other tumours and, finally, since the mode of growth of the cells in tissue culture is typically tumour like In the course of years the virulence of this line has increased during its many passages and the tissue is now able to become established in different mice even without previous X ray irradiation It will be pointed out later that the nature of the inoculated tissue has thus undergone a change which distinguishes it from the tissue found in the spontaneous leukæmias, a change the nature and depth of which we do not know

TRANSMISSION OF MOUSE LEUKÆMIA IN INBRED STRAINS

Whereas the spontaneous development of leukæmia in mice was previously observed only occasionally, the use of inbred strains provided opportunity for studying the leukæmic diseases in large numbers of animals The inbreeding of strains of mice has been developed particularly in the U S A and after a certain number of years of inbreeding such strains display a comparatively high incidence of leukæmias of spontaneous occurrence These inbred strains have also made it possible to obtain regular takes in experimental transmission with far more certainty than before leukæmia —like most tumours—proving to be readily transplantable into animals of the same inbred strain as that in which the line originated

is different from the tissue in the first passage and from the tissue in spontaneous cases When to this is added the fact that the leukaemic cases seen in the passages of a line are composed of cells foreign to the host, it is clear that there are wide differences between spontaneous cases of leukaemia and those produced by transplantation, especially in the later passages of a line

MacDowell and Richter (1930) subsequently published a report of a new, similarly inbred strain ('StoLi'), and in 1931 one of a third strain (strain 89) The former is distinguished by being almost free from leukaemia, which has been observed only in 1 3 per cent of the animals Moreover, the mice of this strain are not susceptible to inoculation with material from for example, strain C 58 Similar strains have been described by McCoy Hill (1930), Slye (1931) and Dobrovolskaïa Zavadskaïa (1932*b*)

Mercier and Gosselin (1931 *a*, *b*, 1932) also reported a strain of mice in which they re transmitted passages of a lymphosarcoma that originated in a mouse of the same strain Some of the inoculated animals developed changes resembling leukaemia

In 1932 Seibold, Rathbone and Furth reported the first experiments with a stock of mice in which a spontaneous case of lymphogenous leukæmia could be transmitted by inoculation to other animals in the same stock but not to animals of other stocks An inbred strain Ak in which lymphogenous leukæmia occurred commonly and myelogenous leukæmia rarely, was later bred from these stocks (A R and S) In contrast to this spontaneous myelogenous leukæmia was common in strain Rf but lymphogenous rare Finally, both forms appeared in strain S, side by side with atypical cases (Barnes and Sisman 1939)

Numerous studies have been carried out with the mice of these strains, some of which illustrate the various

found in a rise in the number of takes (where this is not 100 per cent from the first passage) and in a progressive shortening of the lives of the animals, as the number of passages increases The greater virulence is also shown by the ability of the particular line, during later passages, to take in animals belonging to strains which were resistant to the earlier passages

How this increase in virulence is to be explained is at present debatable One theory is that the material inoculated in the first passage, whether spontaneous tumour tissue or leukæmic blood or tissue, consists of a mixture of more and less virulent cells During passage a selection is supposed gradually to take place, whereby only the most virulent cells continue to grow, the less virulent dying out This theory is not considered probable by MacDowell and his co workers, in view of their immunisation experiments, which will be discussed in a later chapter

Bittner (1935 1939*a*) tried to explain this phenomenon by assuming that transplanted cells are gradually changed, mutating during passage He emphasised that, when the tumour tissue changes its character in this way during a number of transplantation passages, the change is always towards a tendency to require fewer genes from the host animal in order to take A tumour has even been seen to become gradually non specific and to take in mice of many different strains

Whatever may be the explanation of such increase in virulence it is a fact that the tumour or leukæmic tissue in question changes simultaneously with the increase in virulence for it is only by a change in the tissue itself that the virulence can increase Whether the change is genetic or of some other nature, or whether it involves a change in the degree of differentiation, we do not know for certain, but we do know that the highly virulent tissue from the later passages in a line

negative results, and dried material in which the cells had been killed was incapable of conveying the disease

The conclusion to be drawn from these and other experiments is that the disease can be transmitted only by living cells and that no 'agent' can be separated from the latter

Furth and Kahn (1937) demonstrated that mouse leukaemia can be transmitted to healthy animals by means of a single cell injected intravenously, although they were successful in only a few cases (three out of sixty five mice in strain S 2 and two out of thirty two mice in strain AKf 5) No transmission was obtained with cell free substance The observation that generalised leukæmic changes may appear after inoculation with only one cell is important, for it shows that there is nothing, theoretically, against the assumption that the widespread changes in the organs, and also the blood changes, in leukaemia are the result of the neoplastic transformation of a single cell at some place or other in the body with subsequent dissemination The general ised changes therefore, need not of themselves signify that there is a neoplastic transformation uniformly spread through the whole lymphatic or myeloid system in spontaneous cases of leukæmia

Experiments in which a take has been obtained with one cell show how careful one should be in assessing transmissions obtained with apparently cell free material Furth Seibold and Rathbone (1933) and Richter and MacDowell (1933) found that the number of inoculated cells and the life of the animal were in inverse ratio, and that the number of cells inoculated and the percentage of takes were in direct ratio (MacDowell, Taylor and Potter, 1934)

Furth Tuggle and Breedis (1938) investigated the ability of material irradiated with X rays to take; they found that such material is never active after irradiation

features of the pathological cells, others the inter relationship of the ways in which the various forms of leukæmia express themselves, the factors that control the spontaneous occurrence of leukæmia have also been examined so far as is possible

All investigators agree that the various leukæmic processes must be regarded as in the nature of a neo plastic change in the blood forming tissues, and that pure leukæmias, whether leukæmic or subleukæmic, and leukæmias associated with tumour formation—lymphosarcomata, myelocytomata or chloromata, as well as myeloblastomata—are different forms of expres sion of the same neoplastic process, no hard and fast boundaries can be defined between these different forms

Furth, Seibold and Rathbone (1933) concluded that the proliferating cells in lymphogenous leukæmia are not lymphoblasts but pathological malignant lympho cytes with limited power of maturation It is similarly held that *the proliferating cells in myelogenous* leukæmia are pathological neoplastic myelocytes (Furth, 1934c, 1935a, Barnes and Furth, 1935 36) Potter also expressed this opinion as a result of his studies with the strains of MacDowell, Potter, Victor *et al* (1937)

ATTEMPTS TO DEMONSTRATE A VIRUS IN MOUSE LEUKÆMIA

The question whether a cell free virus capable of transmitting the disease can be demonstrated for mouse leukæmia as in the case of fowl leukæmia, has received much attention from investigators

Richter and MacDowell (1933) tried in many ways to obtain takes with cell free material or with killed cells They found however, that no successful transmission of mouse leukæmia could be achieved with their strains unless intact living cells were employed Attempts at transmission with filtrates always gave

theory of the presence of a virus in the leukæmic cells under consideration—which moreover in the various experiments came from lymphogenous, myelogenous and atypical (megakaryocytic) cases of leukæmia—it is not impossible that there is nevertheless, a virus of mouse leukæmia As Engelbreth Holm and Frederiksen (1938*b*) pointed out, the hypothesis that such virus was, for instance so labile on oxygenation that it was inactivated far more rapidly than the known virus of fowl leukæmia might explain the failure to demonstrate it if every possibility of oxygenation during the experiment were not excluded Working on this assumption, these authors tried to transmit leukæmia from Furth's strain Aka to young animals of the same strain, in which cell containing material gave 100 per cent of takes Animals in which leukæmia had developed were killed with potassium ferricyanide to inhibit possible oxygenating processes before autopsy which was performed in a closed chamber filled with nitrogen The enlarged lymph nodes were taken out, minced, and emulsified in a cysteine solution The tissue emulsion was transferred to a small closed chamber which was exhausted so as to remove any possible remnant of oxygen in the emulsion and was then filled with nitrogen After this the cells were separated off by repeated centrifuging for long periods in a closed flask filled with nitrogen A little cobalt sulphate was then added to the clear liquid thus obtained so that a cobalt cysteine reduction system was formed After it had stood for a suitable length of time this liquid was injected subcutaneously into young animals of the same strain In the course of nine such experiments the development of typical leukæmia was obtained in 36 out of 179 animals at a period in their lives when spontaneous leukæmia is never ordinarily seen, most of the animals dying of leukæmia at the age of 2–4 months

with 15,000 r, a dose which, as has been mentioned, is ineffective with the virus of fowl leukæmia Rapid *freezing* to $-30°$ C destroyed the ability of the cells to take in Breedis, Barnes and Furth's (1937) experiments, but the cells can tolerate $-70°$ C if they are *frozen slowly* Breedis and Furth (1938) called attention to the remarkable fact that material frozen slowly retained its vitality and ability to take for so long a period as 440 days at $-70°$ C

A successful transmission with material preserved for a long time at a low temperature means only that the cells are still alive, it cannot be said to indicate the presence of a virus This is clear from experiments in which such frozen cells were irradiated with X rays in doses (4000 r) which do not affect known viruses after which they were found unable to take Dried tissue, glycerinated tissue and Berkefeld filtrates did not produce takes (Barnes and Furth 1937)

Rask Nielsen (1938) tried to demonstrate a possible virus in Krebs's transplantation line of myelogenous leukæmia in mice this line, as already mentioned, was originally regarded as lymphogenous but was reclassified as myelogenous by Rask Nielsen (1936) and Kaalund Jorgensen (1936) On the basis of the fact that pathological cells are destroyed by irradiation with doses (5000 10 000 r) of X rays which do not destroy the known viruses Rask Nielsen thought that it might be possible to prove the existence of such an agent by inoculating freshly irradiated cells, the latter would die some time after inoculation but they might first be able to transmit the hypothetical virus to the new organism About a hundred mice were inoculated in this way, ten of which were irradiated beforehand with 300 r to lower their resistance Not a single take was obtained

Although all these results directly contradict the

theory of the presence of a virus in the leukæmic cells under consideration—which, moreover, in the various experiments came from lymphogenous, myelogenous and atypical (megakaryocytic) cases of leukæmia—it is not impossible that there is, nevertheless, a virus of mouse leukæmia As Engelbreth Holm and Frederiksen (1938*b*) pointed out, the hypothesis that such virus was, for instance, so labile on oxygenation that it was inactivated far more rapidly than the known virus of fowl leukæmia might explain the failure to demonstrate it, if every possibility of oxygenation during the experiment were not excluded Working on this assumption, these authors tried to transmit leukæmia from Furth's strain Ak a to young animals of the same strain, in which cell containing material gave 100 per cent of takes Animals in which leukæmia had developed were killed with potassium ferricyanide to inhibit possible oxygenating processes before autopsy, which was performed in a closed chamber filled with nitrogen The enlarged lymph nodes were taken out, minced and emulsified in a cysteine solution The tissue emulsion was transferred to a small closed chamber, which was exhausted so as to remove any possible remnant of oxygen in the emulsion and was then filled with nitrogen After this the cells were separated off by repeated centrifuging for long periods in a closed flask filled with nitrogen A little cobalt sulphate was then added to the clear liquid thus obtained so that a cobalt cysteine reduction system was formed After it had stood for a suitable length of time this liquid was injected subcutaneously into young animals of the same strain In the course of nine such experiments the development of typical leukæmia was obtained in 36 out of 179 animals at a period in their lives when spontaneous leukæmia is never ordinarily seen, most of the animals dying of leukæmia at the age of 2 4 months

Extensive control experiments showed that if care were not taken to prevent oxygenation no takes resulted, and that it was probable that the liquid injected was cell free

It thus looked as though cell free transmission of the disease had been attained There may, however, be another explanation of these results It might be argued that cases of leukæmia observed in the inoculated animals ought not to be considered as 'transmitted cases' but as cases of spontaneous origin accelerated by the treatment given to the animals, and thus appearing earlier than usual Although it cannot yet be explained how this acceleration may take place, other experiments indicate that the appearance of spontaneous cases of the disease really can be accelerated * *These* problems will be considered in greater detail in Chapter VIII

DEVELOPMENT OF LEUKÆMIA AFTER TRANSMISSION

It seems clear from the studies of Richter and MacDowell (1930) and of MacDowell and Richter (1931) that the leukæmic changes which occur after inoculation with cell containing material are caused by dispersion and multiplication of the inoculated cells, without the co operation of the host's own tissues Hence it is evident that if susceptible animals are inoculated with material from a spontaneous case of leukæmia, the resulting leukæmias should show the same characteristics as the original case As has already been mentioned several times, cases of spontaneous leukæmia vary very considerably Some are leukæmic, others aleukæmic,

* MacDowell Potter *et al* (1939) state that their attempts to demonstrate the transmission of mouse leukæmia with acellular material extracted in the presence of the cobalt cysteine reduction system gave negative results On the other hand in one of their experiments it appeared that the cobalt cysteine system alone without the tissue extract induced (or accelerated) the development of leukæmia This finding however, could not be repeated

some have infiltrations especially in the liver, others are characterised by enlargement of the lymph nodes, others again are lymphosarcomatous rather than truly leukaemic. And the cases produced by inoculation do, in point of fact, to some extent retain the same features as those of the disease in the donor animal. In this connection, Richter and MacDowell described four lines (A, H, I and E), each of which retained its own characteristics through a large number of passages. The white cell picture in the blood was different for the various lines, but fairly constant for each of them, leukaemic in line I, subleukaemic in H and generally normal in A and E. Ascites was observed in line A, rarely in H and I. The extent of infiltration in the liver and kidneys varied in the same way. Similarly, Victor and Potter (1933) and Victor and Wintersteiner (1934) found during their investigations into the metabolism of leukaemic lymph nodes—of which more details are given below—that these lines varied also as regards metabolism, though each was relatively constant individually.

Similar differences in various lines were reported by Furth, Seibold and Rathbone (1933) who, however, noted that the site of inoculation exercised a certain influence on the distribution of the inoculated cells. The morphology of the cells in certain lines in the experiments of these authors, was so characteristic (vacuoles, azurophil granules, nuclear form and pattern, size of cell, maturity, etc.) that a particular line could often be identified by it.

MacDowell and Richter (1931) showed that the genetic constitution of the tissue of such an established line was constant, but that variant lines might display differences. The influence of the genetic factor on the result of transmission experiments will be further discussed in Chapter VII.

METABOLIC CHANGES IN LEUKÆMIC TISSUE

It has been mentioned that Victor and Potter (1933) demonstrated certain metabolic abnormalities in tissue from the leukæmic lymph nodes of animals with spontaneous leukæmia belonging to the inbred strain C 58 referred to above They found that the oxygen consumption of the leukæmic tissue, as measured directly in Ringer's solution with 0 2 per cent glucose, was generally higher and never lower than that of corresponding normal tissue The aerobic glycolysis, measured by Warburg's method, was also generally found to be increased *The anaerobic glycolysis was increased in* every case The results of these first experiments seemed to be clear and simple, and the authors stated that the changes in metabolism corresponded to those found by Warburg in benign tumour tissue It was emphasised, however that tissue could not be recognised as normal, benign, or malignant from its oxygen consumption and glycolysis alone, since the changes in question are not specific to the different types of tissue

Later research showed that the position is far more complicated than had at first been supposed Victor and Wintersteiner (1934) found differences in the metabolism of various lines originating from one and the same spontaneous case and transmitted through genetically uniform mice The external conditions in which the leukæmic cells grow influence their metabolism, and the conditions under which their metabolism is measured are also of great importance Victor and Potter (1934) *showed that two inbred strains of mice (C 58 and StoL1),* the metabolism of whose normal lymphoid tissues was closely similar, affected inoculated leukæmic substance from two lines (I and M liver) differently The cells of line I showed considerably lower rates of aerobic and anaerobic glycolysis when they grew in strain StoL1 than

when they grew in strain C 58, while the oxygen consumption was found to be higher. After a few passages through one strain the leukæmic line concerned was transferred to the other strain, whereupon the metabolism became changed accordingly. This means, then, that the genetic constitution of the host can influence the *metabolism of the inoculated cells*. The *experiments* also indicate that the metabolic changes are due not to fundamental changes in the cells, but only to 'a determining environmental factor'.

In 1935 Victor published an account of a new constant pressure differential volumeter by which it became possible to measure the metabolism in pieces of tissue weighing less than 1 mgm. By use of this apparatus, it was shown that the environmental conditions during the actual measurement are of great importance. Victor and Potter (1938*c*) examined cells of the same line in normal serum, in the serum of immunised animals and in the serum of leukæmic animals. The surprising fact emerged that, while the metabolism of the cells was the same whether they were examined in normal serum or in the serum of immunised animals, it *was distinctly changed when they were examined in the* serum of leukæmic animals. Thus the serum of immune animals had no influence on the metabolism of line I whereas leukæmic serum had a powerful influence. The oxygen consumption of the cells was greater than the excretion of carbonic acid in leukæmic serum, while the contrary was the case *in normal serum*. Leukæmic serum also seemed to inhibit the anaerobic glycolysis of the cells, which was 20 per cent. less in leukæmic than in normal serum.

Subsequent investigations by Victor and Potter (1938*d*) have shown that these differences are due, in part at least, to the fact that mouse leukæmic serum has a markedly lower content of sugar (about 70 mgm

per 100 c cm) than that normally found in mice (about 200 mgm per 100 c cm) Such an enormous difference must obviously greatly influence the metabolism of cells, as measured by glycolysis and the respiratory quotient This discovery led, among other things, to investigations into the question whether the administration of glucose to leukæmic mice had any influence on the course of the disease It proved to have none

In explanation of the surprisingly low blood sugar content of the serum *in mice with leukæmia*, Victor and Potter stated that the leukæmic cells, *formed rapidly in enormous numbers, possess the capacity for oxidising glucose like normal cells but also show far greater rates of glycolysis* The investigations of these authors (1935 *a*, *b*) into the metabolic conditions in the tissues of animals with spontaneously developing leukæmia are of even greater interest They examined the metabolism of the lymph nodes of mice of various ages, some of which belonged to strain C 58 (in which 90 per cent of the animals develop leukæmia) and some to strains in which spontaneous leukæmia is rare or does not occur In the young animals (six to eight weeks old) some difference was certainly found in the oxygen consumption, but there was no clear difference in the aerobic or anaerobic glycolysis in the strains examined, the consumption of oxygen decreased with age in all the strains In the case of the older animals (six to ten months old), on the other hand a distinct difference was found between those from leukæmic and those from other strains the lymph nodes of the older animals from strain C 58 showing higher aerobic and anaerobic glycolysis than those from the young animals, whereas the glycolysis of the leukæmia free strains decreased with age In other words, metabolic changes in the direction of malignancy were found in the lymph . of animals of the leukæmic strain as age increased

not in the leukæmia free strains These changes were demonstrable before morphological signs of manifest leukæmia were observed, the latter did not occur until about a month later

Victor and Potter admitted, however, that, although manifest signs of leukæmia could not at first be found on microscopic examination, leukæmic cells might none the less be present in the tissues This view was supported by the results of transmission experiments which were stated in a later report (1938 *b*) to have been positive Analogous results were reached by Kaalund Jorgensen (1936) who also noted that tissue from organs histologically free from metastases was capable of transmitting leukæmia These findings could be explained by the assumed presence even of single cells which, as Furth has shown are able to transmit the disease

MacDowell Potter Victor *et al* (1936) stated that, when the metabolism begins to change in these lymph nodes, certain abnormal changes in the reticular cells can be demonstrated and that these may perhaps be regarded as the initial cellular manifestation of leukemia Such changes are observed not only in the lymph nodes but also in the liver and spleen From this the authors drew the conclusion that the origin of leukemia is not restricted to lymphnodes, it may arise in a wide range of positions and organs'

As we have said, the inoculation of single cells may give rise as in Furth s experiments to the development of general leukæmic changes without any apparent co operation on the part of the host s own cells Potter and Richter (1933), as a result of their studies came to the conclusion that there is no sign of increased pro duction of the host s own lymphatic cells after the inoculation of lymphogenous leukæmic tissue By examining serial sections made at different intervals after

inoculation they were able to watch how the dispersion and infiltration into the various organs extended from the inoculated cells, the participation of the host's own cells in the development of the leukæmic processes not being observed at any period Investigations such as these can be quoted to support the theory that in spontaneous leukæmia a locally defined transformation of single cells at some point in a lymph node or the bone marrow may be reckoned as the very beginning of the disease, dispersion from these points to other organs being regarded as a form of metastasis On the other hand, the observations of widespread reticular cell changes, referred to above, indicate that it might just as legitimately be assumed that widespread systematic formation of cells in many organs is the beginning of the disease

Finally Victor and Potter (1938*b*) investigated the metabolism of normal lymphatic tissue in animals that had been inoculated with leukæmic tissue After intraperitoneal inoculation with an emulsion of leukæmic spleen or lymph node pronounced metabolic changes were observed in the lymph nodes before it was possible to demonstrate infiltration by the cells inoculated The anaerobic glycolysis was decreased, while the oxygen consumption and the aerobic glycolysis remained unchanged *Inoculation of normal splenic* tissue in control experiments was not followed by any changes in the metabolism of the lymph nodes

These experiments were performed to throw further light on the question of a possible participation of the host organism s own cells in the development of the leukæmic process after the inoculation of leukæmic material Previous experiments have shown, as already mentioned, that there is no reason to assume anything of the sort On the contrary it seems clear that all the changes in a transmission line are produced by the

cells inoculated, which, moreover, retain their own characteristics throughout the passages This being so, it might be expected that the lymph nodes in a host organism would preserve their normal metabolism, even after the inoculation of leukæmic material, until they were infiltrated by the inoculated cells, a change in metabolism would then occur and the tissue from the lymph nodes would thereafter show the increased aerobic and anaerobic glycolysis typical of leukemic tissue Quite unexpectedly, however it was found that the anaerobic glycolysis of these lymph nodes undoubtedly decreased during the period when there was no trace of infiltration After the inoculation of strain C 58 mice with line I tissue, there was a fall in the anaerobic glycolysis of 25 per cent on the first day after inoculation, and after inoculation with line A tissue a fall of 20 per cent on the second day after the inoculation Control experiments showed that these changes must be regarded as a specific effect of the leukæmic material

Victor and Potter concluded from these studies that the inhibiting effect on the glycolysis of normal cells may be presumed to act apart from the leukæmic cells in the inoculum and that an 'inhibitor must circulate in the blood and tissue fluids They expressed the view that the inhibition of the anaerobic glycolytic activity of uninfiltrated lymphoid tissue of mice with transmitted lymphatic leukæmia depends on the following factors (1) specific response to individual transmission lines, (2) interval after inoculation and/or quantity of leukæmic tissue in the host, and (3) a humoral factor which is either a product of leukæmia cells or the resultant of a reaction between host and leukæmia cells

It is hardly possible as yet to judge the full importance of these findings It appears from the experiments quoted that light has been thrown on many of the conditions affecting the factors on which the development

of leukæmia after inoculation with leukæmic material depends, but, though these have been to some extent explained, there are still very many obscure points There are also in some of the experiments hints of phenomena about the nature and significance of which we can only guess

On the other hand, the numerous experiments in the transmission of mouse leukæmia to healthy mice by inoculation with leukæmic tissue make it evident that there are profound differences between the transmitted and the spontaneous disease

The pathological changes in the transmitted cases in all probability depend primarily or entirely upon the multiplication of cells foreign to the host organism Whether 'leukæmia' occurs after inoculation, however depends just as much on the genetic constitution of the host as on that of the inoculated tissue The hereditary factors on which susceptibility depends in such cases are different from those concerned in the spontaneous development of leukæmia The metabolism of the inoculated tissue will often be changed on its introduction into the new organism, but it will still retain a large number of its own characteristics In transmission experiments, when the genetic constitution of the inoculated tissue and that of the host are different, reactions occur which are never met with in cases of spontaneous origin (For further details see next chapter)

These facts do not however, lessen the great importance of such transmission experiments for the very behaviour of the inoculated leukæmic cells in the different conditions offered by the new host has taught us a great deal about the properties of the cells that we could never have learned from the study of spontaneous cases alone Nevertheless, the existence of important differences between spontaneous and transmitted leukæmia must always be borne clearly in mind

Recent investigations by Lawrence and others with radioactive phosphorus (P^{32}) are of interest in that they show the similarity in another respect of leukæmic cells to those from undisputedly malignant tumours (lymphosarcoma and mammary carcinoma) Whether the metabolism of P^{32} is identical with that of normal phosphorus is immaterial from this point of view, as there is a striking difference between normal and malignant cells, in the manner in which they metabolise P^{32} According to Jones, Chaikoff and Lawrence (1940), the 'phosphorus activity' of lymphoma and lymphosarcoma was at least twice that of normal lymph nodes, while Tuttle, Erf and Lawrence (1941) found that leukæmic infiltration in mice of the Strong A strain inoculated with leukæmic cells, was accompanied by a remarkable increase in the uptake and retention of radioactive phosphorus by the nucleoprotein and acid soluble fractions of the liver, spleen and lymph nodes

PART III

THE ROLE OF HEREDITY IN ANIMAL LEUKÆMIA

CHAPTER VI

HEREDITY IN THE SPONTANEOUS DEVELOPMENT OF LEUKÆMIA

CASES of spontaneous leukæmia have occasionally been encountered in related animals—as in related human beings—a finding which has called attention to the hereditary aspect of the disease

THE SIGNIFICANCE OF HEREDITY IN FOWL LEUKÆMIA

No observations of the kind just mentioned have been made as regards birds but it has been stated in various quarters (see Chapter I) that fowl leukæmia occurs with particular frequency in certain breeds *e g* White Leghorns Jarmai (1932) tried to approach the problem experimentally for which purpose he collected twenty one eggs of hens that had been injected with leukæmic virus Twenty of these eggs hatched out successfully, but leukæmia did not appear in any of the birds Experiments of this sort however, cannot really be expected to shed light on the possible inherit ance of the disease for it is obvious that the genetic constitution of the inoculated hens can hardly be thought to be influenced by inoculation with virus The study in question merely showed that the virus was not trans mitted to the egg in this particular instance

It was mentioned on page 27 that Wall (1938) thought that he had observed a decrease in the number of cases of leukæmia in some flocks of fowls the leukæmic birds in which were excluded from breeding

THE SIGNIFICANCE OF HEREDITY IN CATTLE LEUKÆMIA

As early as 1896 Hartenstein expressed the opinion, based on his observation of leukæmia in a cow and in its mother, that lymphogenous leukæmia might be hereditary Eichhorn (1918) and Share Jones (1927) reported one case each in which the disease had been found in a cow and in its daughter Schäper (1938) collected reports of twenty three such cases He re produced a pedigree in which a cow and a bull, which had both died of leukæmia, had two calves both affected with the disease One of the latter, a bull was the father of three calves all of which died of leukæmia Lockau (1933) observed several cases in which the progeny of leukæmic cows had leukæmia Czymoch (1938) gave an account of eight cases, one of which concerned two leukæmic calves from the same cow His statement that sixteen out of twenty leukæmic cows bred in a particular district were the offspring of one and the same bull which itself died of lymphogenous leukæmia is of special interest Both Czymoch and Schaper claim to have observed many cases of leuk æmia in herds in which a particular bull was used for breeding purposes the occurrence of the disease suddenly ceasing on removal of the bull They recommend the exclusion of leukæmic animals and their progeny and Schaper demands that the grandparents as well as the parents of a bull be free from leukæmia if the animal is to be used for stud purposes

While many cases of leukæmia in parent and off spring have thus been observed it must be remembered that the disease has a tendency to appear enzootically in cattle This might, indeed, be regarded as the result of a hereditary disposition since many of the cows in a herd are often related but an enzootic based

on factors other than heredity could obviously affect many of the animals in a herd

This problem cannot be solved on the data at present available, and no major investigations aimed at elucidating the matter are yet forthcoming Meanwhile, however, modern research on the undoubted heredity of mouse leukæmia leads to the fairly obvious assumption that a hereditary disposition may also be of significance in the leukæmias of other animals, including man

THE SIGNIFICANCE OF HEREDITY IN MOUSE LEUKÆMIA

The hereditary aspect of mouse leukæmia has been studied in many quarters, but the experiments carried out by MacDowell Richter and their co workers to elucidate this involved problem are outstanding

Dobrovolskaia Zavadskaia (1932*b*) worked with various strains of mice and noted that far more cases of leukæmia and related diseases, such as lymphosarcoma occurred in one of these strains (III) than in four others Fifteen out of sixty seven males and fourteen out of seventy six females of strain III developed leukæmia, whereas the disease was observed in only a small percentage of the animals of the other strains The author thought that this difference could hardly be explained in any other way than by assuming that it was caused by a hereditary disposition

Slye (1931) maintained a large colony of mice, and leukæmia appeared in many of the strains all of which were cancerous she also mentioned other strains which were quite free from leukæmia As a result of her studies she felt justified in concluding that leukæmia, like other neoplastic diseases is inherited as a simple Mendelian recessive whereas the non malignant property is inherited as a simple Mendelian dominant MacDowell (1935) and Richter and MacDowell (1935)

reviewed Slye's statements, and were of opinion that her conclusion could not fairly be drawn from the material she employed, more especially since the results of cross breeding had not been examined and only a small part of the total number of animals was included in the report Finally, it would appear from Slye's paper that the somatic condition of the mice, *i e* whether the animal had leukæmia or not, was always taken as an expression of its genetic constitution This is not always so, as will appear from what follows

The first definite information about conditions determining the inheritance of leukæmia in mice became available when the experiments on MacDowell's strain C 58 were published (MacDowell and Richter, 1935) It has already been mentioned that from the eighteenth generation of brother sister matings 90 per cent of the animals in this strain had leukæmia, very largely of the lymphogenous type Actually, 450 out of 543 cases were lymphogenous (19 being lymphosarcomata) 6 were myelogenous and the remainder doubtful (55 probably lymphatic and 3 probably myeloid') The animals in this strain were considered to be almost identical genetically as regards the development of leukæmia That 10 per cent of them did not develop leukæmia does not mean that their genetic constitution was different from that of the 90 per cent, for the progeny of the 10 per cent of non leukæmic animals developed leukæmia just as frequently as the progeny of the 90 per cent of leukæmic animals The failure to develop leukæmia in the 10 per cent must therefore be attributed to the presence or absence of extrinsic, non hereditary factors This provides a beautiful illustration of the theory that manifest leukæmia in an animal may be and in most cases certainly is due to co operation between intrinsic genetic factors and extrinsic non hereditary factors In certain cases

the hereditary factor may be so predominant that leukæmia can be developed with the aid of little or no external influence, as in strain C 58 On the other hand, extrinsic factors—as yet unknown—may perhaps in certain hypothetical cases be supposed to cause leukæmia without the co operation of hereditary predisposition

This may be assumed to have occurred in another of MacDowell and Richter's (1935) strains—StoLi Only 1 3 per cent of cases of leukæmia appeared in this strain from the fourteenth to the twenty ninth generation of brother sister matings The strain in question is further distinguished by its very high incidence of mammary carcinoma which is only very rarely seen in strain C 58

EXTRA CHROMOSOMAL HEREDITY IN MOUSE LEUKÆMIA

Experimental cross breedings were now established between the two strains C 58 and StoLi Since both strains were considered to be genetically homogeneous as regards the occurrence of leukæmia, the descendent F_1 generation must also be assumed to be genetically homogeneous in this respect An interesting feature now appeared—in that the frequency of leukæmia in F_1 depended on whether C 58 males were crossed with StoLi females or *vice versa* In the former case 42 5 per cent of 106 animals developed leukæmia whereas the offspring of C 58 females and StoLi males developed leukæmia in 61 9 per cent *i e* 19 4 per cent more This difference is statistically significant (4 5 × P E)

The experiment shows firstly that the non hereditary factors play a relatively greater part in the manifestation of leukæmia in F_1 than in strain C 58 It also demonstrates that 'leukæmic' or 'non leukæmic' need not be a direct expression of an animal s genetic constitution Secondly it shows very remarkably that

among the non chromosomal influences which co operate in determining whether leukæmia develops or not, a principal factor seems to be transmitted from the mother to the offspring Since the percentage of cases of leukæmia is approximately the same for the two sexes in F_1, there can be no question of sex linkage

Back crossing from F_1 to StoLi showed the following features When F_1 females were crossed with StoLi males, 46 5 per cent of the offspring developed leukæmia, whereas only 19 8 per cent of the progeny of F_1 males and StoLi females developed leukæmia—another instance of the maternal non chromosomal influence

That the animals in F_1 were genetically homo geneous was apparent from the fact that their off spring developed leukæmia in the same percentage, whether the F_1 parent was leukæmic or not

From these experiments MacDowell and Richter concluded that ' the predisposition to leukæmia and related diseases in mice of strain C 58 is specifically heritable The state of affairs in F_1 indicates that the genetic difference between strain C 58 and strain StoLi is not a single recessive Mendelian gene as assumed by Slye The frequency of leukæmia in F_1 and in the back crossings 'is roughly correlated with the propor tion of total heredity from strain C 58 Non chromo somal variables play a role that grows increasingly important as the proportion of total leukæmic heredity is reduced ' Among the non chromosomal influences an important one is transmitted by the mother It is not possible from these experiments to establish any definite rules of heredity for the disease

It is not clear how the extra chromosomal ' heredity that is transmitted from the mother to the offspring is to be understood MacDowell and Richter were most inclined to assume that there is a 'cytoplasm heredity They examined the possible explanation that the

mother's milk might be of influence, but concluded that it could not, since mice of strain C 58 which were nursed by mothers of non leukæmic strains developed leukæmia to the same extent as other C 58 mice, and StoLi mice did not develop leukæmia after being nursed by C 58 mothers These experiments, however, are discussed only briefly Similar (unpublished) experiments were carried out by Frederiksen and Engelbreth Holm, who examined mice of Furth's strain Aka which were nursed by mothers from a strain in which leukæmia did not occur, but in which cases of breast cancer were seen About 50 per cent of the animals so nursed that attained the age of eight months (sixty animals) developed leukæmia which is not appreciably different from the percentage in Aka mice of the same generations nursed by Aka mothers In addition, mammary carcinoma occurred in seven of these sixty animals—a remarkable finding, since this disease is observed only very rarely in strain Aka No case of leukæmia was observed in the mice of the non leukæmic strain which were nursed by Aka mothers

In MacDowell and Richter s experimental crossings with strains C 58 and StoLi a corresponding non chromosomal maternal influence on the development of mammary carcinoma was found in F_1, the daughters of StoLi mothers developing breast cancer far more frequently than the daughters of C 58 mothers The difference was 39 7 per cent which was stated to be 7 7 times the probable error

Recent research on the subject of mammary carcinoma has shown the very important role of mother's milk in the development of this form of tumour (see for example Bittner, 1939*b*) Thus Hagedoorn and Hagedoorn (1937) observed that whereas 83 2 per cent of the females nursed by mothers of the same strain developed mammary carcinoma, only 4 9 per cent of

the females of the same strain developed this disease if they were nursed by females of another strain To this it can now be added that such a change in the frequency of carcinoma in a strain may occur suddenly MacDowell and Richter (1935) observed that the frequency of mammary carcinoma in strain StoLi suddenly fell at the nineteenth generation from 50 6 to 4 7 per cent That a fact of this sort cannot, *per se*, explain observations such as the Hagedoorns' appears from Bittner s (1939*a*) experiments Mammary cancer occurred in only 7 4 per cent of 95 animals which themselves belonged to an inbred strain (A) in which 83 6 per cent of 1093 animals developed mammary carcinoma, but which had been nursed by mothers belonging to a strain with a low incidence of tumours (strain C 57 black and CBA) Further breeding revealed the remarkable fact that cancer developed in 66 2 per cent of the offspring of females which, in spite of nursing by non cancerous mothers, had developed mammary cancer, as compared with only 9 8 per cent of the offspring of females which, after nursing by non cancerous mothers, proved to be free from cancer A number of exceptions to this rule had, however, been observed The experiments were carried further analogous 'nursing experiments' being performed with hybrids between strain A (cancerous) and strain B (C 57 black, low cancerous) Here, too the extra chromosomal maternal factor was very evident as can be seen from Fig 37

From these and other experiments there can be no doubt of the existence of a 'breast cancer producing influence' which is transmitted through the milk of cancerous mothers, but, as Bittner says, 'The nature of this 'influence'—hormone, chemical, or virus—has not been determined '

Carcinoma of the lung also occurred in Bittner's

strain, but its frequency was not affected in the experiments, by nursing by different mothers Bittner's work has been discussed in detail, in order to illustrate the difficulties which may be encountered in investigating hereditary factors in mice

Certain experiments have clearly shown a maternal non chromosomal heredity' in leukæmia, but it has been demonstrated from two quarters that this feature

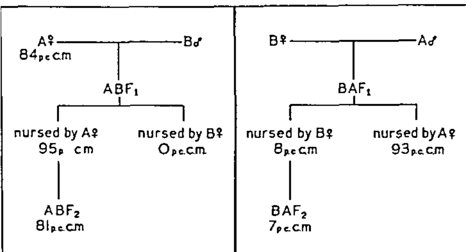


FIG 37—Extra chromosomal factors in inheritance of carcinoma mammæ Breast cancer percentages (c m) in control and fostered reciprocal F_1 hybrids and F_2 generation mice (Redrawn from Bittner (1939) *Amer Journ Cancer* xxxv 95)

cannot be explained by influence through the mother s milk which in one of the strains concerned, has a distinct influence on the development of mammary carcinoma

It would thus seem that we are faced with two different sorts of maternal non chromosomal influences on the offspring one through the mother s milk (mammary cancer) and another possibly 'cytoplasmic heredity which influences the development of leukæmia Neither of these two modes of influence has yet been explained It has already been indicated that Richter and

MacDowell's experiments did not permit a clear definition of the process of heredity as regards leukæmia in mice It can only be said that leukæmia is not inherited as a simple recessive Mendelian gene

Little, Murray and Cloudman (1939) investigated the conditions of heredity affecting the occurrence of non epithelial tumours in strains C 57 and dba—altogether 2247 animals Strain C 57 had a relatively low incidence of tumours, only 64 non epithelial tumours (and 10 epithelial), of which 37 were 'lymphoblastomata', being observed among 568 animals The dilute brown (dba) was a high breast tumour strain, with about 50 per cent mammary carcinomata and only a small number of tumours of other sorts, of the few non epithelial tumours observed, over 80 per cent were leukæmic When these two strains were crossed the distribution of non epithelial tumour formations (*i e* principally leukæmias) among the hybrids was examined It was clearly shown that the rules for the inheritance of non epithelial tumours are different from those for mammary carcinoma It would appear, however, from these studies that there ' might be a maternal or extrachromosomal influence which affected the relative non epithelial tumor forming potentialities of various tissues or organ systems "

As in Richter and MacDowell's experiments it is impossible to explain the inheritance according to simple Mendelian rules If a type of Mendelism is involved, it includes multiple factors and a marked influence of the environment Thus the heredity of mouse leukæmia has not yet been elucidated, but it is agreed that inherited factors play an important and in certain inbred strains a decisive, part Furthermore, extrinsic, non inherited factors are also important, and among these the factors that are transmitted through the mother as non chromosomal influences are of particular

interest Finally, the action of external influences on the animals, as will be discussed more fully in a later chapter, is sometimes decisive and leads to the development of leukæmia in not a few mice of strains in which only a very few cases occur spontaneously (see Chapter IX)

It has been pointed out by Gorer (1937 *a*, *b*) that various strains of mice differ genetically in their resistance to the milk borne agent of mammary cancer Bittner has analysed this problem, and thinks it likely that the difference in resistance between the A (high cancer) and C 57 (low cancer) strains is determined by a single gene, susceptibility being dominant (Bittner, 1939, 1940)

A large number of facts become explicable if the extra chromosomal agent be regarded as a virus The sudden change in the mammary tumour incidence of the StoLi strain, mentioned above, might be due to variation of a virus It is known that the effect of breeding upon tumour incidence varies greatly from strain to strain, in some strains virgin females are almost free from mammary tumours, whilst the incidence in breeders is high, in others there is little difference between virgins and breeders It has always been difficult to explain this fact along purely genetic lines, and here again the difference might be due to variations between the viruses carried by different strains

In the case of leukæmia there does not appear to be any agent transmitted in the milk This is not surprising on the hypothesis that the causative agent of mouse leukæmia is a virus, for whereas a virus stimulating mammary tumour formation must gain access to the mammary gland and its presence in the milk is not unexpected, in a disease like leukæmia different conditions would prevail and the agent might be transmitted through the placenta and be unable to survive in mammary tissue

CHAPTER VII

THE SIGNIFICANCE OF HEREDITY IN TRANSMITTED MOUSE LEUKÆMIA

MacDowell, Richter and their co workers carried out very comprehensive investigations into the influence of hereditary factors on susceptibility to inoculated leukæmic material

Whether a mouse will prove susceptible to inoculation with leukæmic material depends on the relation between the genetic constitution of the mouse and that of the material inoculated The material used for inoculation in MacDowell and Richter's (1930) studies was kept constant, *i e* tissue from a single line was used in all the experiments The inoculation of over 1200 animals of the inbred strain C 58 produced *100* per cent of takes of line I (see also MacDowell 1936), whereas the inoculation of strain StoLi did not produce a take in any animal The animals of F_1 generation, a cross between C 58 and StoLi provided *100 per cent* of takes (*177* animals) (Fig *38*) *There were takes in 79 5 per cent of the animals in* F_2 *Back crossing from* F_1 *to the susceptible strain* C *58 produced animals that* were 100 per cent susceptible and back crossing from F_1 to strain StoLi resulted in animals that were 48 2 per cent susceptible The genetic experiment showed that, *in a purely statistical sense there was no significant* deviation from the expectation if only one gene were concerned Careful analysis showed however, that this explanation was insufficient The authors therefore concluded that while a single gene is of primary importance in determining susceptibility, subsidiary

factors must also be involved, since certain individuals carrying the particular gene may occasionally survive, while, on the other hand, a few animals succumb in its absence This somewhat puzzling finding may be explained along the lines advocated by Gorer (see p 154

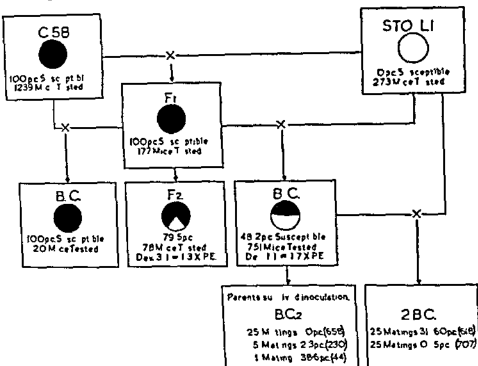


FIG 38—Inheritance of susceptibility to inoculation of mice of strains C 58 and StoLi with leukæmic cells of line I There is evidence that the susceptible and non susceptible strains of host differ by one pair of Mendelian genes with dominance of susceptibility (Redrawn from MacDowell (1936) *Amer Journ Cancer* xxvi 89)

and Gorer 1941) If the possibility of eliciting an immune response be determined by iso antigenic factors present in the inoculated cells but absent from the host it would appear that line I leukæmic cells contain one powerful antigen and perhaps one or two other factors that are poor antigens The fact that not all iso antigenic factors are equally potent is stressed by Gorer

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cases Susceptibility to the leukæmias that had occurred in the F_1 and F_2 generations was also investigated The behaviour of these leukæmias in transmission experiments was the same as that of Ak leukæmias

The experiments showed that susceptibility is inherited as a dominant character but it was not possible to decide from this material whether one or two dominant genes are responsible for the susceptibility

The course and duration of the disease were not affected by the genetic constitution of the host Moreover the experiments provide clear evidence that there is no question of a susceptibility to transplanted leukæmia in general as susceptibility to Ak leukæmia and to Rf leukæmia are two entirely different inherited factors which are not allelomorphic Schweitzer and Furth claim that the hereditary factors which determine susceptibility to transplanted leukæmia are different from those which determine whether the transplantation of normal tissue will be successful but their arguments on this point are not wholly convincing

The experiments just quoted show indisputably that hereditary factors play an important part in the susceptibility of the mouse to inoculation with leukæmic tissue but the mechanism of the hereditary process can be clearly defined only as regards an inoculum of a given line since the genetic constitution of the tissue inoculated is also one of the factors that decide whether a take will result Mice of an inbred strain that is susceptible to inoculation with tissue from one line may thus quite well be resistant to leukæmic tissue from another line

IMMUNITY REACTIONS TO TRANSMISSION AND THEIR DEPENDENCE ON HEREDITY

It has already been mentioned that immunity reactions may develop in the host when mice are inoculated with leukæmic material The occurrence of these

Under favourable conditions the subsidiary antigens of line I may stimulate a successful defence in animals unable to respond to the main antigen, by reason of its presence in *their normal tissues* *Other animals* apparently fail to respond to the main antigen in the absence of the subsidiary antigens

Other experiments (Richter and MacDowell, 1935) were less definitive, inoculation with tissue from another line (A) into mice of the same strain and its hybrids giving results which might be taken to indicate that more than one gene (presumably from two to seven) decides susceptibility Thus, on the one hand, the co operation of several hereditary characters must be reckoned with, and on the other, extrinsic factors may, in certain circumstances, play a part and make the picture con siderably more obscure

Schweitzer and Furth (1939) performed similar experiments with other strains They worked with two strains—Ak, in which about 70 per cent of the animals developed lymphogenous leukæmia spontaneously, and Rf, in which only 1 or 2 per cent developed leukæmia, of a myelogenous or monocytic type Susceptibility to inoculated material from spontaneous cases of leuk æmia in these two strains was examined, partly in animals of the two strains and partly in animals of F_1 (cross mating Ak × Rf), F_2 and F_3, and also in animals bred by back crossing F_1 and strain Ak or Rf

Taken as a whole, the results confirm those of *MacDowell and Richter mentioned above, the* tissue from the lines emanating from strain Ak producing 100 per cent of transmissions in Ak and F_1, but not in Rf The lines from Rf produced 100 per cent of transmissions in Rf and F_1, but not in Ak The lines from Ak produced 100 per cent of transmissions in back crosses F_1 × Ak Back crosses F_1 × Rf produced animals in which lines from Ak took fairly well and lines from Rf in 100 per cent of the

MacDowell, Taylor and Potter (1934) inoculated mice of strain C 58 with leukæmic tissue from their line I and obtained 100 per cent of takes in 2925 animals The amount of tissue usually employed for inoculation contained about 80 million cells If fewer cells were used the course of the disease was prolonged, while inoculation with less than 200 cells failed to produce a take It appeared that animals which survived inoculation with such small doses were resistant to larger doses, and it was found possible to immunise 114 mice by means of repeated inoculations with increasing quantities of cells so that they survived the ordinary 'standard inoculation' of about 80 million cells

This observation gave rise to a larger series of investigations, which have yielded most interesting results

MacDowell, Potter and Victor (1935) showed that the immunisation obtained was not specific to the combination line I—strain C 58 employed, but could be reproduced with other strains and lines These experiments, moreover presented the apparent paradox that *it is possible to immunise against a highly virulent line but not against one which is less virulent, and also that it is only possible to immunise against material that has undergone several passages* Thus it was found that mice immunised against a highly virulent line produced 50 per cent of takes when inoculated with tissue from a less virulent line These features will be more fully discussed later

Potter and Findley (1935) investigated histologically the fate of leukæmic cells inoculated into immunised animals They showed that immediately after inoculation the cells grow as in non immunised animals, though considerably more slowly This is indicated, for instance by the fact that only from 0 3 to 1 per cent of the cells are found in a state of mitotic division as against from

reactions is very closely connected with the relation of the genetic constitution of the tissue inoculated to that of the animal receiving it These features will be discussed in the present section

One extreme of the series of results obtainable in such transmission experiments is represented by the inoculation of tissue from an animal belonging to an inbred strain, *e g* strain C 58, into other animals of the same strain, with 100 per cent of takes, indicating that there is no essential genetic difference between the tissue inoculated and that of the host The other extreme is represented by experiments aimed at inoculating rats with mouse leukæmic tissue, this has not as yet been successful, because, among other reasons, antibodies are developed in the rat against the genetic ally foreign tissue inoculated, which sooner or later dies In this case, however, it may be presumed that factors other than purely immunisatory co operate Between these extremes there are the attempts to trans mit mouse leukæmia to mice whose genetic constitution is different from that of the tissue inoculated The result of these transmissions will depend on the differ ence between the genetic constitutions It has already been mentioned (Clemmesen, 1938) that resistance can be lowered by irradiating the animals before inoculation, the immunisation processes being retarded so that a take is attained in spite of the usual difficulties It has also been mentioned that the heterologous transplantation of mouse leukæmia into rats may be attained in this way

It is now known that immunisation can occur even when leukæmic tissue is inoculated into mice of the same strain, which would appear to mean that there is no complete genetic identity in different animals, even in those of a strain that has long been inbred It must be borne in mind, however, that no strain of animals is ever more than approximately pure (Haldane 1936)

given injections of normal C 58 splenic tissue all died after the inoculation of leukaemic tissue This question has not yet been submitted to further investigation, and the nature of the 'passive immunity' obtained in such experiments is difficult to understand

IMMUNISATION AGAINST MOUSE LEUKÆMIA WITH EMBRYONIC TISSUE

Besides producing immunisation by treatment with increasing doses of leukæmic cells, MacDowell, Taylor and Potter (1935) succeeded in immunising mice against leukæmic tissue by the injection of normal living embryonic tissue from other strains The immunity thus obtained however, was said in later experiments to have displayed essential differences from the immunity given by leukæmic cells The first attempt was made with mice of strain C 58, these, as has been mentioned several times, are 100 per cent susceptible to leukæmic tissue from line I, against which strain StoLi is more resistant (in later passages of line I the tissue took in about 50 per cent of the cases) By injecting C 58 mice intraperitoneally with a suspension of StoLi embryos minced in 0 5 c cm saline per embryo, immunity was obtained This was evident from the fact that inoculation with leukæmic tissue (line I) in the usual way six to nine days later produced no takes in 93 mice, whereas 104 control mice all died Treatment of 75 animals with C 58 embryos did not have a similar effect, as was also to be expected On the other hand, hybrid embryos from C 58 mothers by StoLi fathers were able to immunise mice The results of these various experiments clearly illustrate the dependence of the immunity upon the genetic relation between the immunised animals and the embryos by means of which the immunity was produced Immunisation of about half the animals used was obtained in other

4 to 9 per cent in tissue inoculated into non immunised mice In the course of the first few days degenerative changes occur in the peripheral cells of the transplant These changes extend rapidly and soon convert all the inoculated tissue into chromophilic debris At this point phagocytes appear which remove the debris fairly rapidly Not until after this process is complete are lymphocytes from the host seen in the connective tissue where the inoculated tissue had been, and they soon disappear At the end of six or seven days no trace of the inoculum remains The host's lympho cytes do not, therefore, play so important a part in the destruction of the inoculated tissue as has been supposed by many authors Clemmesen's experiments (1938) with other tumours in hetero transplantations led him to the same conclusion, namely, that the lymphocytes of the host are of minor importance in the destruction of tumour tissue implanted into immune animals The cells so implanted seem to die and disappear before any cellular reaction in the host sets in How else the destruction of the implanted cells is to be explained has not yet been shown No harmful action of the serum from immune animals on the tumour cells against which they are 'immune' has been demon strated (MacDowell Potter and Victor, 1935) It is also a fact that tumour cells can be cultivated *in vitro* in immune plasma

The immunity to leukæmic tissue which is obtained by the methods described above can be transmitted passively from the immune animals to other mice by the subcutaneous injection of 0 1 c cm of minced splenic or liver tissue Potter, Taylor and MacDowell (1938) treated thirty one mice of strain C 58 in this way, and three days later inoculated tissue from line I (standard dose) None of the animals developed leukæmia Sixteen untreated animals and seventeen that had been

which have not been published in detail—that two factors are operating The resistance on the third day is regarded as a direct effect of the embryonic tissue or its decomposition products on the inoculated cells The later resistance is then the true 'immunity' induced by the embryonic tissue There seem, indeed, to be three distinct processes that can prevent a take 'the third day action' of embryonic tissue, the later (temporary) immunity induced by embryonic tissue, which, again, is clearly different from the third process—the permanent immunity conferred by leukæmic cells in increasing doses

Although these findings are still somewhat obscure, they have been discussed in detail because other attempts to immunise with embryonic tissue have given remarkable results

After inoculation with leukæmic tissue the usual picture of generalised leukæmic changes in the liver, lymph nodes, spleen, etc, was rarely observed in the mice treated with embryonic tissue, though localised subcutaneous lymphosarcomata occurred These tumours were observed in 101 animals Some of them were single, some multiple, and in some cases they were found to be combined with generalised changes From the fact that further transmission from 31 of these cases to untreated animals produced leukæmic changes of the usual type it appears that the same morbid process is involved as is usual in the line employed The changed nature of the cells was retained only in one case the altered form of expression appearing repeatedly in five passages The authors conclude from these experiments that the lymphosarcomata observed should be regarded as 'partially suppressed leukæmia' and that the embryo treatment does not confer the capacity to destroy inoculated leukæmic tissue (as in the immunity given by leukæmic cells), but only to suppress

experiments (MacDowell, Potter and Victor, 1935) in which different embryos were employed In the experiments first quoted there was an apparent correlation between the immunising power of inoculated embryonic tissue and the natural resistance of the animal supplying it *Experiments with hybrid embryos*, however, showed this to be fallacious, and that the *results* depended *only* on the ability of the tissue to stimulate an immune response in the animal receiving it

The immunity induced by the injection of embryonic suspensions shows many differences from that obtained by treatment with leukæmic cells The latter, as mentioned above, can be transmitted passively to other mice, whereas the former could not (Potter, Taylor and MacDowell, 1938) It has also been mentioned that cells inoculated into animals which have been immunised with leukæmic tissue at first multiply a little, though slowly, after which they die and are resorbed Such initial growth of the inoculated tissue cannot be demonstrated in mice that have been immunised with embryos But the differences between the two methods of immunisation appear still more striking when the duration and strength of the immunity obtained are compared (MacDowell Potter, Victor, Taylor *et al*, 1936) While the immunity given by leukæmic cells is permanent, that from treatment with embryonic tissue is temporary and variable It was found for example, *that on inoculating* embryo immunised mice with leukæmic tissue at various periods after treatment *little or no immunity could be* demonstrated for the first two days By the third day all the mice were resistant, but on the fourth partial resistance only could be traced After this the immunity increased evenly for three weeks Two months later it diminished again Immunity has not been attained by using dead embryonic tissue

The authors conclude from these experiments—

tissue nine died from leukæmia before the first untreated one died Treatment with embryonic tissue thus showed this most remarkable effect—*the growth of slightly virulent cells (from spontaneous cases) was accelerated, while the growth of highly virulent cells (from long transplanted lines) was restricted, in some cases completely* (MacDowell, Potter, Victor, Taylor *et al*, 1937) In conformity with this, MacDowell, Potter and Taylor (1937) found that treatment with embryonic tissue, in the form of monthly injections into animals of the inbred leukæmic strain, resulted in an acceleration of the spontaneously developed disease Whereas 90 per cent of the untreated animals of the same strain developed leukæmia, all of the sixty animals treated with embryonic tissue died of that disease at a rather younger age than the controls

These results show how vast a difference there is between the cells in spontaneous cases and those in the long transplanted cases Their interpretation raises important issues It is possible that they are to be explained by the fact that no strain of animals is ever more than approximately pure On the other hand it is difficult to interpret the finding that the progress of the inoculated disease in earlier passages is accelerated by a process which induces immunity in later passages MacDowell *et al* do not accept the explanation that inability to obtain immunity during the earlier passages is due to a failure to immunise the animal against cells of its own genetic constitution, and that success in later passages is due to the selection of a highly virulent and atypical type of cell

POTTER S THEORY

Potter (MacDowell Potter Victor Taylor *et al*, 1937) attempted to explain the findings just mentioned upon the following lines From the observation that,

the leukæmic cells to a greater or less extent—a suppression which is generally temporary but may be permanent

THE EFFECTS OF IMMUNISATION ON THE DEVELOPMENT OF LEUKÆMIA

In the experiments already quoted, constant host animals (C 58) and inoculated tissue (line I) were used and the genetic constitution of the embryonic tissue used was varied In other experiments the inoculated leukæmic cells were varied, tissue from various lines or from different passages in a single line being used, the host and the embryonic tissue used for immunisation remaining constant It was found that a given treatment with embryonic tissue which immunised mice against tissue from the later passages of a line showing great virulence, was considerably less effective against inoculation with tissue from the same line's first passages or with tissue taken direct from a spontaneous case, which, in ordinary transmission experiments, proved to be far less virulent than tissue from the line's latest passages Takes are obtained in all untreated mice by inoculation with tissue from spontaneous cases and from late passages of a line, but the disease develops more slowly in the former case When the animals are treated with embryonic tissue the leukæmic tissue from spontaneous cases takes constantly, whereas the highly virulent, long transplanted cells no longer take It was also found that the cells from such spontaneous cases as showed specially slight virulence in control experiments grew more rapidly in animals treated with embryonic tissue than in the control animals Untreated mice died on the average, 41 days after inoculation with 'spontaneous' cells whereas those treated with embryonic tissue died as early as 32 days after it Of twenty animals treated with embryonic

resulted in a more rapid and frequent development of spontaneous leukæmia in these animals than in untreated animals of the same strain, are reminiscent of Engelbreth Holm and Frederiksen's (1938*b*) experiments, in which a very similar result was attained by the injection of tissue from leukæmic lymph nodes (p 115) It is still not possible to express an opinion as to whether there is any question in these two experiments of an analogous or uniform process Such process, according to Potter s theory, should consist in an ability of the injected embryonic tissue or extract of tumour tissue to inhibit differentiation of the tissues which in these strains tend to undergo malignant conversion, and thereby to promote the manifestation of the disease since inhibition of the differentiation would mean an increase in malignancy

It seems not improbable that two different processes are involved in the experiments of MacDowell Potter Taylor *et al*, and that these two processes now and then occur simultaneously in the experiments thereby making the picture more difficult to interpret The one would be an inhibition of differentiation resulting in increased malignancy and an acceleration of the morbid process the other an immunising process against transplanted tumour tissue, with consequent inhibition or cessation of growth of the inoculated tissue

There is however, also the theoretical possibility that the apparently contradictory results—acceleration of the spontaneous disease and of the growth of inoculated tissue in a first passage and retardation or stoppage of the growth of the tissue in the later passages of the same line—are produced by one and the same action namely inhibition of cell differentiation and perhaps also of other cell functions

If Potter s theory be accepted that inhibited differentiation is an essential feature of malignant cells, it

in every case of leukæmia, there are cells at all stages of differentiation, with relatively more undifferentiated cells than are found in normal blood, it is assumed that an essential feature in the malignancy of the cells is a decrease in the rate of normal differentiation Malig nant lymphocytes differentiate more slowly than normal lymphocytes, but the differentiation proceeds steadily Now it appears that a certain speed of differentiation is typical in each case, and is apparent in the relation between differentiated and non differentiated cells as found in the leukæmic tissue The degree of differentia tion is in inverse ratio to the virulence of the tissue in question , the slower the differentiation, the greater the proportion of primitive non differentiated cells and the greater the virulence

This theory, though not improbable, certainly fails to offer an immediate explanation of the involved features of the immunisation experiments described However, the problem may seem less insoluble if the term ' virulent cell ' be replaced by ' immature cell ' and the results of the experiments be expressed as follows—*the more immature (non differentiated) the cells in an inoculum, the more easily are they influenced by the processes in duced by immunisation* Nevertheless, the acceleration by the same immunising process of the relatively more differentiated cells remains just as obscure, unless it be assumed that the immunisation can inhibit the differ entiation of the inoculated cells, which would explain the sudden increase in malignancy An assumption of this kind might, perhaps, similarly explain the increase in virulence during passage in a line in which, according to Potter s hypothesis, the inoculated cells might be assumed to be more and more restricted in their differ entiation and thus progressively more malignant

The experiments mentioned above, in which the injection of embryonic extract into mice of strain C 58

The experiments were carried out with animals from two strains, one susceptible and the other refractory to the sarcoma in question, and with crosses and back crosses between these two strains A report on the work, published in 1938, also included an account of transplantation experiments with leukæmias which had occurred in these strains The experiments showed that the specificity, from the point of view of immunisation, displayed by the tumours and leukæmias which occur in inbred strains is presumably due to the mosaic of antigen characters which they possess, and not to any abnormality in malignant tissue By means of detailed experiments including immunisation studies, titration of iso antibodies formed, and absorption tests it was shown that immunity against transplanted tumour or leukæmic tissue is to be regarded as essentially dependent on iso antibodies analogous to those which appear in response to other cells of the organism, leukæmic cells seeming even to contain more iso antigen of one of the types examined than do erythrocytes An indication was found however, that malignant cells can in some cases grow in spite of antigenic differences Further, the possibility is constantly admitted of an antigenic difference between tumour tissue and the tissue from which the tumour originates, but these differences are not usually indicated by the formation of antibodies in transplantations

Although several factors are still obscure, Gorer's interesting results open a possibility of a simpler explanation than has yet been available of the extremely complex features which characterise immunisation experiments with transplantable tumours and leukæmias

seems not unreasonable to suppose that an influence capable of inhibiting differentiation may quite well produce increased malignancy It might, however, be expected that a greater influence in the same direction, involving an increased inhibition of differentiation and possibly of other functions, would ultimately cause the death of the cells, as an expression of the total inhibition of one of their functions

Arguments of this kind inevitably come to mind in the course of attempts to solve certain problems of modern tumour research One may instance the action of X rays, and perhaps also that of carcinogenic hydro carbons, which can not only produce tumours but also, in certain circumstances, inhibit or even stop the growth of the tumour that they themselves have caused These facts may perhaps be legitimately compared with the two apparently contradictory actions of the immunising processes in the experiments mentioned above

But considerations such as these are remote from the path of exact investigation and belong rather to the realm of speculation

THE NATURE OF THE IMMUNISING PROCESSES

The nature of the immunising processes observed on the inoculation of leukæmic and other tumour tissue has been elucidated through a series of beautiful experiments by Gorer (1937*a*, 1938) This author, who in previous studies had demonstrated iso antigens in erythrocytes from three different inbred strains of mice, showed, in 1937 by inoculation experiments with a sarcoma which had arisen in one of these strains, that susceptibility to this tumour was dependent on at least two dominant genes, one of which appeared to be identical with the gene which determines the appearance of the iso antigenic factors previously demonstrated

PART IV

ATTEMPTS TO PRODUCE LEUKÆMIA EXPERIMENTALLY

Two principal methods have been employed in attempts to produce leukæmia experimentally in animals The influence of hæmotoxic poisons has been used to study the question whether the continued maximal regeneration attainable by exposing the body to the effects of these poisons can be made to change into the abnormal super regenerative conditions which seem to be characteristic of leukæmia The other method frequently used is based on the assumption that leukæmia is a tumour of the hæmopoietic tissues it consists essentially of attempts to influence these tissues by means of carcinogenic substances which have already been shown capable of producing tumours in other tissues

The interpretation of the results obtained by both these methods is often difficult since in almost all cases the investigator is faced with the problem of deciding whether the changes produced are to be regarded as non specific leukæmoid reactions or true leukæmic processes

CHAPTER VIII

EXPERIMENTAL PRODUCTION OF LEUKÆMIA IN FOWLS

LEUKÆMOID REACTIONS

It has already been mentioned that the hæmopoietic system of fowls is extraordinary labile, so that the most varied influences may result in violent reactions with the development of a greatly altered blood picture Moore's (1897) 'infectious leukæmia' in fowls was referred to in Chapter I, and it was mentioned that this was an instance of a typical leukæmoid reaction to an infection with *Bacterium sanguinarium* Mention was also made of the enormous leucocytosis (see Fig 39) which is so prevalent in tuberculous fowls that it has even given rise to the assumption that fowl leukæmia is only a form of fowl tuberculosis

Kasarinoff (1910) investigated the influence of many blood poisons on pigeons and fowls He examined the effects of hæmotoxic substances such as cholic acid, ricinoleic acid, croton oil and soap, anæmia causing poisons such as toluylenediamine, pyridine, saponin, etc, and finally leucoblastic poisons such as canthari dine nuclein, guanine etc He observed very peculiar blood pictures several times during his experiments—for instance a veritable myeloblastic congestion in the blood of a fowl treated with pyridine, toluylenediamine, and pyrogallol The same picture was seen after treat ment with ricin No more detailed mention however, need be made of Kasarinoff's results, since the reactions were non specific in all cases and always disappeared when the treatment was interrupted, provided the birds



Fig. 39.—Blood of tuberculous fowl with leukæmoid reaction. About 300 000 leucocytes per c mm were present, the great majority being normal pseudo eosinophil leucocytes. Just below the middle of the field are a lymphocyte and a blood platelet. × about 1500

survived No information was given in these studies as to tissue changes in the birds

Other investigators, too, have produced fairly severe non specific blood changes which have, in some cases, been regarded as leukæmic Gohs (1934), for example, injected normal fowl tissue which had been kept for some time in glycerol into his test birds, but there are no grounds for the assumption that leukæmia was produced in these experiments

Furth (1931*c*) examined the possibility of producing erythroleukæmia by repeated bleeding and by the administration of pyridine, ricin, and toluylenediamine His experiments illustrate the unusually marked ability of fowls to regenerate blood, since, even after severe blood loss, and after treatment with poisons causing anæmia, these birds can regenerate the total amount of blood in the course of a few days In these circum stances a very large number of erythroblasts certainly appeared in the blood, but the ability of the cells to mature was preserved in every case and the organs displayed no leukostasis

Jarmai and Balo (1938) produced severe anæmia, accompanied by considerable leucocytosis (with many myeloblasts), by means of Maretin (*meta* tolylsemi carbazide) but the anatomical changes typical of leukæmia did not occur Strangely enough, when an emulsion of organs from these birds was injected into healthy fowls, similar blood pictures developed in two out of three Here also, however, no changes in the tissues were found Further 'transmission' was not successful

Anatomical changes reminiscent of those found in spontaneous leukæmia, were in fact, not observed in any of the above experiments, and the reactions were of a temporary character so that they must be classed as non specific and leukæmoid

EXPERIMENTS WITH CARCINOGENIC SUBSTANCES

Thomsen and Engelbreth Holm's (1931) experiments are more difficult to assess Assuming leukæmia to be a tumour, they injected carcinogenic tar into the bone marrow of sixty two fowls, six months to one year old From 0 02 to 0 05 c cm of tar was injected every fifth day, through a fine hole drilled upwards in the upper end of the tibia Eleven of the birds developed a fairly considerable leucocytosis—up to 100,000 per c mm —and anæmia to 10 per cent of hæmoglobin, but pronounced leukæmic blood pictures were not seen The blood changes might well be explained as an indication of non leukæmic reaction to the tar, although it is surprising that the majority of the test birds did not react with these changes On the other hand, the tissue changes in the eleven birds bore a surprising resemblance to those of leukæmia The following cases which appeared to be identical with myelogenous leukæmia, mixed myelogenous erythroleukæmia and anæmic erythroleukæmia respectively, were of special interest

One fowl died with slight anæmia and severe leucocytosis (106 000 leucocytes per c mm) after treatment for five months The liver was greatly enlarged (see Fig 40) weighing 118 g (normal weight about 35 g) and closely resembling the liver in spontaneous cases of leukæmia The spleen was also greatly enlarged though to a less degree Microscopic examination revealed a considerable infiltration of myelocytes into the enlarged organs and into the kidneys (Fig 41) The marrow too presented the same picture as that seen in spontaneous myelogenous leukæmia

A second fowl was killed because of severe anæmia (hæmoglobin 17 per cent) after treatment with tar for four months Among 46 000 leucocytes per c mm 3 per cent of myelocytes were observed and there were typical basophil erythroblasts in the blood The spleen was distinctly enlarged



FIG 40 —Liver spleen and kidney of fowl with experimental leukæmia after injections of tar into bone marrow Considerable enlargement of liver and moderate enlargement of spleen



FIG 41 —Section of the kidney shown in Fig 40 Interstitial myelocytic infiltrations are seen × about 300

(9 g as compared with the normal 1 2 g), the liver was only slightly enlarged Microscopy revealed moderate myeloid infiltration and a considerable leukostasis of basophil erythroblasts (see Fig 42) The bone marrow (Fig 43) was just like the marrow in spontaneous mixed myelogenous erythroleukæmia No mature elements were observed among the basophil erythroblasts, which were found conglomerated in the capillaries of the organs There were numerous mitoses in these cells Apart from the fact that the blood changes were less pronounced, the histological changes in this bird could not be distinguished with certainty from the changes in spontaneous cases

A third fowl displayed very similar changes

The fourth fowl had extreme anæmia (hæmoglobin <10 per cent) after treatment with tar for two months Some basophil erythroblasts with many mitoses were seen in the blood There were 5 per cent of myelocytes among the leucocytes (14 000) The organs were not enlarged, but, histologically, punctate conglomerations of basophil erythro blasts were found throughout the capillaries The case was highly reminiscent of a spontaneous case of anæmic erythro leukæmia, but differed in that there were only slight changes in the bone marrow

Whether the changes are to be classified as non specific reactions or as experimentally produced leuk æmia can hardly be decided with certainty Trans mission from two of the cases to nineteen chickens in all was tried, and a case of aleukæmic myelogenous leukæmia developed Although this case is unlikely to have been of spontaneous origin, because the bird was so young, it does not seem to be sufficient to prove the transmissibility of the changes produced There is much to suggest that fowl leukæmia was produced experimentally in these birds, but there is no definite proof

Since these first experimental attempts to produce fowl leukæmia by means of carcinogenic substances

many similar attempts have been reported Sarcomata had been produced in fowls even earlier by means of tar, but these tumours had not been successfully trans mitted in passage

Furth and Furth stated in 1938 that they had previ ously carried out similar experiments in which tar had been injected into the femoral marrow of five chickens Seven months later they observed leukæmia in one of these, with 'vast numbers of primitive white blood cells in the blood' They found 560,000 erythrocytes and 930 000 leucocytes per c mm in the blood Attempts to transmit the disease to twelve chickens were not successful

McIntosh (1933) produced several (thirteen) tumours by injecting tar into the breast muscles of twenty one chickens Most of these tumours were fibrosarcomata and fibro angio endotheliomata, and in many cases leukæmic changes of different kinds were also found It was possible to transmit four of these tumours with an emulsion of the tumour tissue, and in three cases transmission was successfully effected with Berkefeld filtrates As happened in the original birds, cases with leukæmic changes (not more fully described) were also seen in the transmission passages

These experiments show how closely the experi mental production of fowl leukæmia is connected, on the whole with the experimental production of fowl tumours This however, might be expected from the occurrence, already recorded of tumour production by leukæmic virus in some strains

No detailed mention need be made here of the numerous attempts which have been reported to eluci date the relation between fowl tumours of spontaneous origin and those produced by carcinogenic substances The vital problem which has not been solved in a way meeting with general acceptance is whether a

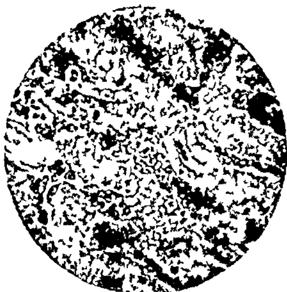


FIG 4[illegible] — Section of kidney of fowl with experimental leukæmia following injections of tar into the marrow Typical leukostasis of basophil erythroblasts in the capillaries × about 500 (*Cf* Fig 14 Figs 40-47 are from Thomsen and Engelbreth Holm (1931) *Acta path S and* viii 1 1)



FIG 43 —Bone marrow of fowl with experimental leukæmia following injections of tar into the marrow The sinuses are dilated and filled with basophil erythroblasts × about 500 (*Cf* Figs 10 and 14)

virus can be demonstrated in the tumours produced by carcinogenic substances, as in the case of spontaneous tumours This is a very difficult question As already mentioned, McIntosh thought he had found Berkefeld filterable 'virus' in the sarcomata and leukæmias which he had produced by means of tar, but several investigators have carried out similar experiments without obtaining takes with cell free material from experimentally produced tumours Thus Mellanby (1934) found that fowl tumours produced by tar and dibenzanthracene are distinct from spontaneous tumours in that they cannot be transmitted by filtrates and their consumption of oxygen is greater than, for instance, that of Rous's chicken tumour I

Peacock (1935) arrived at the same conclusion, but emphasised that the demonstration of a cell free virus is subject to the influence of so many variable and uncontrolled factors that the value of negative results must also be estimated with caution It may even be impossible at certain periods to demonstrate the virus in well known strains of fowl virus tumours by methods which, at other times, allow of its demonstration with ease

McIntosh's first result was confirmed by Haddow (1934), who produced a sarcoma with 1 2 5 6 dibenzanthracene, which could be transmitted through several passages, in which takes were produced both by Berkefeld filtrates and by material that had been frozen and then thawed

McIntosh and Selbie (1939) reported further experiments and mentioned five new sarcomata produced by tar, two of which could be transmitted by means of filtrates They, too, found leukæmic changes in some of the birds In this study the authors referred to similar experiments carried out by other investigators, and they supposed that the various results depended on differ

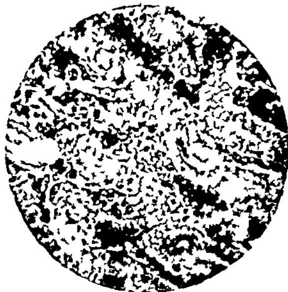


FIG 4 —Section of kidney of fowl with experimental leukæmia following injections of tar into the marrow Typical leukostasis of basophil erythroblasts in the capillaries × about 500 (*Cf* Fig 14 Figs 40-42 are from Thomsen and Engelbreth Holm (1931) *Acta path Scand* viii 1 1)



FIG 43 —Bone marrow of fowl with experimental leukæmia following injections of tar into the marrow The sinuses are dilated and filled with basophil erythroblasts × about 500 (*Cf* Figs 10 and 14)

ences in technique Oberling, Sannié, Guérin and Guérin (1936) painted five fowls with 1 per cent benzpyrene in benzole The skin at the site of painting showed almost no reaction, but typical erythromyeloblastic leukæmia (and an ovarian tumour) developed in one bird two months later The results of transmission experiments (eight birds) were negative

Jarmai and Baló (1938) attempted to produce fowl leukæmia with carcinogenic tar and with benzpyrene A solution of tar in benzole was injected subcutaneously into a large number of fowls Blood and tissue changes very similar to those observed in Thomsen's and Engelbreth Holm's experiments, previously mentioned, occurred seven months later in nineteen birds (21 per cent of those used) Transmission experiments, the extent of which is not known, gave negative results In some other experiments 0 5 c cm of 0 5 per cent benzpyrene in oily solution was injected intramuscularly every fortnight Typical erythroleukæmia developed forty three and sixty days later in two out of ten fowls and this could be transmitted to normal birds in the usual manner One case was transmitted through sixteen passages Jarmai and Baló themselves did not venture to ascribe any importance to this experiment since the results of repeated experiments of the same kind were wholly negative The authors therefore thought that the two cases in question were spontaneous Since the authors themselves emphasised that the cases 'produced' corresponded closely to spontaneous cases under transmission in their laboratory, it seems possible that these were really cases of unintentional transmission from that strain by means of instruments infected by chance This risk exists in every laboratory engaged in research on fowl sarcomata caused by virus as has been emphasised and demonstrated by Begg and Cramer (1929) Simple rinsing

and washing of the mortars and other utensils may prove to be insufficient, the virus perhaps remaining active after such procedure There is no doubt that adventitious factors of this sort may be important Even if all the instruments have been boiled, autoclaved, or otherwise sterilised, such unintentional transmission may occur

Thomsen, Engelbreth Holm and Rothe Meyer (1935) thus observed that five out of twelve fowls in an immunisation experiment (with normal fowl blood) died of typical erythro leukæmia, just as though they had been inoculated with leukæmic blood The donor birds were examined and found to be healthy Attempts to explain the origin of these cases were unsuccessful It is possible that contamination of the hands with leukæmic blood, even after they have been washed, may be enough to produce occasional cases of leukæmia It is worthy of note that this was the only case of the kind which occurred at the institute in question in experiments extending over a period of ten years

Finally, mention should be made of Mottram's (1935 36) experiments in which three out of twelve fowls which had been exposed to powerful irradiation with radium died 1 2 years later of fibrosarcoma, spindle cell sarcoma, and lymphosarcoma, respectively

DISCUSSION OF RESULTS

It is certain that sarcomata have been successfully produced in fowls by the action of carcinogenic sub stances—tar, carcinogenic hydrocarbons and radium (of these experiments, only those in which the tumours produced were related to leukæmia have been mentioned here) It is doubtful, however, whether experimental leukæmia has been produced by similar treatment Leukæmoid blood changes have often been produced, but simultaneous leukæmoid, or perhaps leukæmic, tissue changes have been observed in only a

few cases Transmission experiments have been tried many times Thomsen and Engelbreth Holm had one take in nineteen animals McIntosh transmitted his tumours, which were often combined with leukaemic changes, through several passages, even transmission with Berkefeld filtrate was successful These results were confirmed by Haddow, though they have been questioned by others

Whether experimentally produced sarcomata contain a virus is thus a question which has been the subject of considerable argument Certain studies in which attempts have been made to demonstrate the presence of such a virus have been referred to above In the following experiments an effort to elucidate this problem was made by more indirect means

Andrewes (1936) worked with a fowl sarcoma which was produced by tar and could be transmitted to fowls by inoculation with cellular tumour tissue, though not with filtrates It was capable also, in some cases, of successful transplantation into pheasants, but the inoculated tumour then generally regressed after growing for some time as usually happens in cases of heterologous transplantation In a couple of cases, however, large progressive tumours appeared, and one of these gave precipitin reactions which might be taken to indicate that the tumour in this bird consisted of pheasant cells Furthermore, antibodies which neutralised the virus of the Rous sarcoma No 1 developed in the inoculated pheasants Nothing like this is seen when pheasants are immunised with normal fowl tissue Andrewes therefore concluded from these experiments that the tumour produced by tar contained a virus, although the latter was not demonstrated by transmission experiments with filtrates

Very similar results were obtained by Foulds (1937) A sarcoma which had occurred in a fowl, after three

injections of 1 2 5 6 dibenzanthracene in lard, could be transplanted in passage to other fowls, with increasing virulence Cell free material would not take Rabbits were immunised with material from this tumour, and thereby developed antibodies which neutralised the virus in extracts from the Rous sarcoma No 1 The rabbit serum preserved its virus neutralising effect even after absorption with the tissue of fowl embryos Foulds also deduced from his experiments the existence of a virus in the non filterable tumour, and compared the fact that virus cannot always be demonstrated in these tumours with the fact, referred to previously, that even in the Rous sarcoma No 1 the virus may periodically escape demonstration

A comparable feature is observed in the Shope rabbit *papilloma, caused by a virus in cotton tail rabbits* When this is transmitted to domestic rabbits, tumours are certainly produced, but from them the virus cannot be isolated directly Nevertheless, antibodies to the virus can be demonstrated in these host animals (Kidd, Beard and Rous, 1935)

How the relation between tumours produced experimentally in fowls and those that occur spontaneously is to be interpreted can hardly yet be finally decided Andrewes's dictum must be emphasised, that "non filterability of a tumour is no proof that a virus is not concerned in its ætiology" It must also be stressed that the fact that experimental fowl leukæmias seem to have been transmitted only in rare cases cannot be taken as proof that these diseases are essentially different from leukæmias of spontaneous origin since only some of the latter have proved to be transmissible

CHAPTER IX

EXPERIMENTAL PRODUCTION OF LEUKÆMIA IN MAMMALS

LEUKÆMIAS have been 'produced' experimentally in mice and rats by carcinogenic agents Recent research seems to indicate, however, that such leukæmias should be regarded as spontaneous diseases accelerated by the treatment in question, so that they appear earlier and with greater frequency than is usual in these animals These considerations, which will now be more fully discussed open the much wider question whether it is possible at all to produce tumour formation in animals by carcinogenic agents unless there is a certain con stitutional tendency in the animals to develop tumours

LEUKÆMOID REACTIONS

Leukæmoid blood changes which may sometimes be misinterpreted as a sign of true leukæmia are also known in mammals as in fowls An example of such a typical non leukæmic leukæmoid reaction was that reported by Feldman and Stasney (1937) in rabbits Enormous leucocytoses up to 110,000 and 124 000 per c mm were produced by the injection of tuberculin into eleven tuberculous rabbits Immature elements—myelo blasts ('leukoblasts') promyelocytes and myelocytes were often found among the white blood corpuscles The changes occurred from twenty four to seventy two hours after the tuberculin reaction disappearing and not returning even when the administration of tuber culin was continued this, taken in conjunction with the absence of leukæmic tissue changes, shows the non leukæmic character of the reactions

Similar leukæmoid pictures can be induced in the higher mammals Ludke (1910) produced them in monkeys and dogs by the injection of pyridine, with subsequent infection with streptococci and staphylococci

The findings of Eisler (1937), who investigated the blood changes in rabbits during anaphylactic shock produced with horse serum, were presumably of a similar kind This author continued the administration of the antigen after desensitisation, whereupon a considerable fall in the leucocytes, resembling agranulocytosis, occurred When, however, he performed these experiments with animals previously treated with hydroxyl amine, Eisler found blood pictures which resembled myeloblastic leukæmia Fuller particulars of the changes are not given, so that a detailed assessment of the results is impossible

Leukæmoid reactions are not unusual in mice In these animals it is often difficult to draw a definite boundary line between such non specific reactions and true leukæmia In this connection Parsons's experiments are particularly illustrative In 1935 she reported a transplantation line originating in a sarcoma produced by injections of sodium 1 2 5 6 dibenzanthracene 9 10 endo $\alpha\beta$ succinate In the mice in which the sarcoma grew after inoculation a 'leukæmia' with up to 400,000 white blood corpuscles per c mm, developed simultaneously Myeloid metaplasia was found in the spleen in these animals and the bone marrow was pus like, highly reminiscent of leukæmic marrow In 1936 fresh experiments were reported which made it doubtful whether the changes just described were really leukæmic or more in the nature of a myeloid reaction caused by the growing tumour In any case, it was impossible to separate the tumour from the leukæmoid changes, which were never seen in animals apart from the tumour In a few cases, Parsons (1936 1938) succeeded in transmitting

the sarcoma concerned (line from 'Mal sarcoma 1') by means of filtrates An extract of tumour was prepared in 0 1 0 2 per cent cysteine hydrochloride (to prevent oxidation), and filtered through collodion membranes which retained particles larger than 0 35 0 7 μ Five tumours of the type usual in this line were produced by the filtrate in 39 out of 199 mice These tumours too were accompanied by blood changes as described above

Retransmission of the sarcoma by means of blood from the tumour bearing animals was successful in later studies (1938) in which changes in the lymph nodes of the inoculated animals were also described, these included changes in the reticular cells and even others that were interpreted as sarcomatous They did not seem to be metastatic but were assumed to be due to a non cellular product of the sarcoma Lymphatic hyperplasias not leukæmic in character, could also be seen in the lymph nodes

It is impossible to decide from the descriptions whether these changes in the reticular cells of the lymph nodes were of the same kind as those observed by MacDowell Potter, Victor *et al* (1936) after inoculation with leukæmic tissue and regarded as the initial cellular manifestation of leukæmia occurring simultaneously with the first demonstrable metabolic changes in the tissue (p 121) The American authors assume, with Parsons that the tissue may be influenced by a humoral factor whose nature is unknown

Barnes and Sisman (1939) reviewed a number of cases of myelogenous (myeloid) leukæmia in mice and compared them with non malignant extramedullary myelopoiesis observed as the result of various influences They put forward many differential diagnostic observations which are useful when decisions have to be made as to whether a certain reaction is leukæmoid or

leukæmic As a result of their studies the following criteria were advanced (Table III)

TABLE III

Myelogenous leukæmia	Non malignant extramedullary myelopoiesis
Most of the myelogenous cells are immature	*All stages in the development of myelogenous cells are present*
Erythrogenic foci are absent among the myelogenous cells	Erythrogenic foci are usually present
Megakaryocytes are *few* and present only in the organs (spleen liver and lymph nodes) where they are found in non leukæmic conditions	Megakaryocytes are usually numerous
The myelogenous cells often invade muscle and other non hæmatopoietic tissues	*The cells are non invasive*
The blood usually contains immature myelogenous cells	*The blood is either normal or there is a leucocytosis with numerous mature forms*
The liver is usually enlarged and grey brown	*The liver is usually not enlarged and is brown red*
Most of the lymph nodes are usually enlarged.	*Most of the lymph nodes are usually of normal size*
Hæmorrhages are frequent in the viscera (lungs lymph nodes etc)	Hæmorrhagic manifestations are absent
Condition is transmissible to other mice	*Condition not yet shown to be* transmissible
Condition not shown to be produced *by bacteria*	Condition can be *produced by bacteria*

It is often difficult to disentangle the true facts especially in the case of mice, in which it would seem that extramedullary myelopoiesis may appear physiologically, *e g* in the spleen, *especially in the older* animals Mice react to very many influences with fairly considerable *myeloid metaplasia and leucocytosis* It is evident from the examples reviewed that inoculated tumours and infections of various kinds can release leukæmoid reactions Barnes and Sisman's studies also contain several examples of spontaneous tumours accompanied by myeloid metaplasia and leucocytosis

EXPERIMENTS WITH CARCINOGENIC SUBSTANCES

The first attempts to produce leukæmia experimentally in mammals by means of carcinogenic hydrocarbons date from 1928 Lignac treated white mice with subcutaneous injections of benzene in oil The leucopenia typical of benzene poisoning soon appeared and when the treatment was continued for a long time, many of the animals died Small greyish white nodules in the liver, which perforated the capsule, were observed in three out of the twenty six animals thus treated Lignac described these nodes as sarcomatous and attributed to the cells in them 'myelopotente Eigenschaften' There was no mention of leukæmia in this experiment Brandt (1928) produced similar changes in a rabbit by painting it twice a week for eighteen months with tar dissolved in benzene Systematised extensive proliferation of cells of an obscure nature occurred in the organs The spleen was enlarged and white nodes were found in the kidneys, intestine, suprarenal glands and lymph nodes The bone marrow was not examined Histologically the nodes consisted of undifferentiated malignant cells which had possibly originated in the walls of the vessels There was no question of leukæmia in this case Subsequently Lignac (1933) claimed by giving minute repeated subcutaneous injections of benzene in oil over long periods, to have produced leukæmia and lymphosarcomata in 8 out of 33 mice of a strain ordinarily free from such conditions These experimental attempts to produce leukæmia in animals by means of benzene are of interest in relation to the accumulating, but not yet conclusive evidence that prolonged exposure to commercial benzol may occasionally produce acute or chronic leukæmia in man (For literature see Hunter 1939 and Mallory Gall and Brickley 1939)

Bungeler (1932 *a*, *b*), working on the basis of Fischer Wasels's regeneration theory of the origin of malignant tumours, tried to produce leukæmia in mice by treating them with indol in order to cause chronic indol poisoning He found that tissue metabolism was changed by indol poisoning, the consumption of oxygen decreasing and anærobic glycolysis increasing He compared these conditions with those found in malignant tumours (It must, however be remembered that these metabolic changes, while certainly characteristic of tumour tissue, are not specific thereto)

Bungeler treated 594 mice with two or three weekly injections of from 0 1 to 0 2 c cm of 0 1 per cent indol in water (dissolved by heat) Many of the animals died with hæmolytic anæmia and leucopenia Several months later incipient regenerative processes could be traced and, of 97 animals that survived the treatment for eight months four are said to have developed aleukæmic lymphogenous leukæmia or lymphosarcoma, and thirteen myelogenous leukæmia, of which nine cases were aleukæmic The significance of these experiments is difficult to determine, partly because no information is given as to how frequent spontaneous leukæmia was without treatment in the mice employed, and partly because it cannot be decided whether the cases classified as myelogenous leukæmia were not perhaps entirely or partly cases with a non specific leukæmoid reaction

Bernard (1934 35 36) working on the same basis and with the same technique as Thomsen and Engelbreth Holm in their experiments with fowls (p 161) attempted to produce leukæmia in mice, rats rabbits, guinea pigs cats dogs and monkeys by injecting carcinogenic tar into the bone marrow Various blood changes were observed in these very extensive experiments most frequently erythrocytosis and leucocytosis in which

immature forms occurred The condition developing in rats was particularly studied From 60 to 70 per cent of the animals reacted with an increase in the number of erythrocytes (up to thirteen or fourteen million per c mm) Some of these later developed fairly severe hyperplasia of the marrow, with considerable leucocytosis Apart from this, no definite anatomical changes of a leukæmic nature were observed The changes were classified under many different categories, but no case of undoubted leukæmia was produced Several transmission experiments with varied technique were all negative Bernard himself characterised some of the resulting changes as leukæmia, but there seems to be no reason for assuming that the changes observed in the marrow and blood should be regarded as anything but leukæmoid reactions No similar changes occurred in rats after the injection of tar into their spleens and lymph nodes (1935)

Storti and Storti (1937) repeated these experiments but employed 1 2 benzpyrene (*i e* 3 4 benzpyrene according to more recent nomenclature) instead of tar The experiments comprised 100 rats, each of which had 1 per cent of benzpyrene in oily solution injected into the femoral marrow (1 mg weekly total 8 mg) Twelve out of 75 surviving animals displayed a series of blood changes consisting of anæmia (with many erythroblasts) and marked leucocytosis On the other hand no tissue changes beyond slight myeloid meta plasia were found even in the spleen The authors concluded and certainly rightly, that the changes must be regarded as non specific reactions to a toxic influence Gennaro and Grazia (1937) also employed benzpyrene but used it as a 1 per cent solution in benzene to paint the skin of the backs of fifteen rats Leukæmia developed in two of these animals myelo genous in one and lymphogenous in the other, there

was also a carcinoma at the site of painting According to the description and pictures, these were cases of true leukæmia, but it was not stated whether leukæmia developed spontaneously in the animals of the strain used

Burger and Uiker's (1937) experiments were of a somewhat different nature *On the hypothesis that* carcinogenic substances *of* the methylcholanthrene type, derived from biliary substances, especially cholesterol, *might possibly be formed in the organism*, a series *of* mice was treated with bile Some of the mice were injected with untreated bile, and some with 50 mg of dried gall bladder bile stirred in a little water and 1 c cm of olive oil Twelve mice were injected twice a week with 0 25 c cm of the latter Changes that were interpreted as leukæmic, both myelogenous and lymphogenous, occurred in nine of these animals It is impossible to decide from the report whether the cases were really leukæmic or merely leukæmoid *The* latter is perhaps the more probable explanation, especially since Merlini (1939) observed only leucocytosis and slight tissue changes of uncertain nature in his similar experiments with seventy rats Merlini considered that the changes were not leukæmic

Uher (1938) also investigated the effect of injecting bile into mice, rats and rabbits He treated the animals with bile and tar or with carcinogenic hydrocarbons A series of blood changes *with severe leucocytosis and* anæmia, was observed but no leukæmia occurred The reactions produced were often temporary, since they passed *off even while the treatment was being continued*

Burrows and Cook (1936) injected a 0 3 per cent solution of water soluble 1 2 5 6 dibenzanthracene 9 *10 endo αβ succinate subcutaneously* or intraperitoneally into mice in doses of from 0 2 to 0 5 c cm twice a week Spindle cell sarcomata *developed* in six

out of sixty animals Two died, or were killed, with enlarged lymph nodes and spleens and with pronounced 'leukostasis' in the liver (after 318 and 397 days, respectively) A third mouse, with a large tumour like formation in the thorax, was killed on the 164th day According to the description, the tumour was presumably an enlarged thymus, such as is often seen in spontaneous leukæmia Histologically, it was composed of lympho cytic round cells which infiltrated into surrounding structures, such as the heart Furthermore, the lymph nodes appeared to be enlarged This case was undoubtedly a lymphogenous leukæmia The other two cases were also classified as leukæmia, which was presumably justifiable Burrows and Cook considered that the lesions in these three cases were produced by the treatment and were not spontaneous since corre sponding pathological pictures were not found in the control animals There was no question of leukæmoid changes and there was no concomitant tumour growth, since tumours of other kinds were not found in the three animals One of the spindle cell sarcomata mentioned above was the origin of the line of trans plantable sarcomata which, in Parsons's experiments, were almost regularly accompanied by leukæmoid blood changes and changes in organs These changes were not found in the donor animal

Nearly all the reported attempts to produce leukæmia in mice were carried out with animals of inbred strains, and in many of the cases nothing was known definitely of any tendency on the part of the animals to develop leukæmia spontaneously The results of most of the experiments were negative or doubtful, but leukæmic conditions presumably unmistakable and most prob ably produced experimentally have been observed in a few cases in both rats and mice Typical leukæmia, demonstrated to be transmissible was not, however,

produced in any of these experiments with the regularity which must be considered imperative before the experimental production of leukæmia in mammals can be accepted as an established fact

Convincing results of attempts to produce leukæmia in mice by carcinogenic hydrocarbons have only recently been attained

Lewis (1938) investigated the production of tumours by 1 2 5 6 dibenzanthracene in a strain (C_3H) in which lesions of the lymphatic system did not appear among the untreated animals There were three lymphosarcomata among 100 tumours thus produced, one of which permitted further transmission to other animals of the same strain but not to animals of other strains Tumours of the lymph nodes and enlargement of the spleen in addition to lymphosarcoma at the site of inoculation developed among the animals inoculated with tissue from one of these lymphosarcomata but the blood did not become leukæmic From the fact that further transmission with the blood of these animals produced takes of lymphosarcoma Lewis concluded that the blood nevertheless contained malignant lymphocytes Another of the cases produced by dibenzanthracene revealed the feature so frequently found in spontaneous cases—a large tumour of the thymus with neoplastic infiltration of the hilus of the lung and auricles of the heart The three cases of lymphosarcoma occurred 75 115 and 237 days respectively after the injection of 0 8 mg of carcinogenic hydrocarbon (in 0 4 c cm olive oil)

Dobrovolskaia Zavadskaia and Rouyer (1938) observed similar cases in a strain (XXX) in which the spontaneous development of 'lymphadenomatosis' was never seen It developed however, in two of the 102 mice treated subcutaneously in the axilla with small doses of 1 2 5 6 dibenzanthracene and also in one

out of fifty one mice in which radon tubes had been implanted subcutaneously That four out of thirty nine mice displayed the same pathological picture after infection with *Spirochæta morsus muris* is more extraordinary This feature, and the fact that more tumours are found in some litters than in others, presumably indicate that constitutional factors in this strain play an essential part in the development of tumours, although no observation of leukæmia in untreated animals has been reported

The experiments of Morton and Mider (1938, 1939) with mice of Little's dilute brown strain (dba) are of great interest Spontaneous mammary carcinoma occurs in from 80 to 90 per cent of the breeding females in this strain and tumours of the lungs in less than 5 per cent 'Lymphoblastoma occurs commonly in both sexes between the ages of 650 and 800 days Morton and Mider painted sixty mice of this strain with 0 5 per cent methylcholanthrene in benzene solution once a week, the site of the painting being varied from time to time In this way the development of tumours of the skin caused by painting was avoided The result was that forty eight of the sixty animals succumbed to leukæmia at a far younger age than is usual, all of them dying within 226 days Most of the leukæmic cases were lymphogenous and displayed the same picture as the spontaneous cases in this strain Whether there was a true increase in the number of leukæmic cases cannot be decided, for lack of accurate information about the controls Examination of the blood often revealed up to 300 000 white cells, of which from 85 to 90 per cent were lymphogenous, with many immature forms The earliest symptoms were observed after treatment for only 69 days Experimental transmission from one of the animals resulted in takes in six out of ten others with the development of a tumour at the site of inoculation and general leukæmic changes

produced in any of these experiments with the regularity which must be considered imperative before the experimental production of leukæmia in mammals can be accepted as an established fact

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almost benign, compared with what occurred in the cases produced by tar. The cases of leukæmia in the experimental series corresponded exactly with those arising spontaneously in leukæmic strains, and were undoubtedly typical lymphogenous leukæmias. No 'antagonism' between leukæmia and the development of carcinoma as a result of painting was observed.

In the light of these results there can no longer be any doubt that leukæmia can be produced experimentally by carcinogenic agents, in mice of known genetic constitution. The question immediately arises as to why this was not observed before, since the strains concerned have been used for experiments, and even for the experimental production of tumours, for a number of years. Brues and Marble mention the possibility that the character of the branch of the strain employed by them (Bagg albino) had changed by mutation. This, however, is hardly likely, since analogous observations have become increasingly frequent (see below). A satisfactory reason for the non-recognition of these features hitherto can hardly be given. It certainly cannot be assumed that such leukæmic changes were overlooked, although it must be admitted that they did not fall within the scope of previous carcinogenic experiments in the strains concerned.

The work of Lewis, Morton and Mider and Brues and Marble demonstrates then that leukæmia can be produced in inbred strains in which the disease rarely occurs spontaneously (C3H, Little's dilute brown, Bagg albino). Moreover, Brues and Marble showed that it is not every inbred strain that reacts in this way, since mice of Little's C 57 black strain appeared to be refractory in this respect. To see the problem in its true perspective it is important to discover how inbred strains with hereditary and frequent cases of leukæmia behave under similar treatment.

Thus, in these experiments, the development of spontaneous leukæmia was successfully accelerated by treatment with carcinogenic hydrocarbons

Brues and Marble (1939), who used the Bagg albino strain and Little's black C 57, observed similar conditions in the one, but not in the other, after painting with tar The object of the experiments was to investigate the influence of various diets on the development of tumours caused by painting, but the result threw light on quite a different problem as not infrequently happens in experimental pathology While the various diet groups behaved uniformly, the painting resulted in the development of leukæmia in many of the animals of the Bagg albino strain Four kinds of tar were used, of which only two resulted in the development of leukæmia a feature presumably connected with the greater general carcinogenic property of these two samples, as evidenced also by a greater incidence and earlier occurrence of papillomata at the site of painting

The strains employed were inbred and were both known as being 'free from leukæmia, which is to be taken as meaning that spontaneous leukæmia does not occur in more than 2 per cent of the animals

Leukæmia developed, however, in six out of thirty mice (20 per cent) after painting in the usual manner with a specimen of tar which produced papillomata after treatment for 179 days whilst another tar, which was apparently more carcinogenic and produced papillomata in the course of 84 days, produced lymphogenous leukæmia in twenty out of forty mice (50 per cent) The same kinds of tar produced papillomata only, and no case of leukæmia, in the mice of strain C 57 Leukæmia was found in about 2 per cent of about 1000 untreated animals of the Bagg strain, but the progress of the disease in these cases was very slow,

organs, the picture thus to some extent resembling that of Hodgkin's disease The leukæmic nature of the disease may perhaps be doubted, even the authors calling it 'a leukemia like disturbance with malignant cells resembling monocytes' As already mentioned, similar pictures are seen, though rarely, in untreated animals of strain Rf and, according to the authors, the effect of the treatment was mainly evidenced by the fact that 'the incidence of this disease was greatly enhanced by the treatment with benzpyrene The experiments certainly showed a distinct increase in the number of cases of this condition in the animals treated, and the fact that the disease was of a type that is rare in spontaneous cases may have been due to the method of administering the benzpyrene

Engelbreth Holm (1940) was able to obtain a clearer answer to the question of the extent to which the incidence of a frequently occurring form of leuk æmia may be influenced by carcinogenic agents He used for his experiments the Aka strain of mice, which originated from Furth's strain Ak From 50 to 70 per cent of the animals in this inbred strain that were over eight months old developed lymphogenous leukæmia No spontaneous leukæmia had been observed in animals less than five or six months old Twenty mice of this strain were given subcutaneous injections of 1 mg 9 10 dimethyl 1 2 benzanthracene dissolved in 0 1 c cm of olive oil when they were six or seven weeks old A number of tumours (sarcomata generally spindle celled and squamous celled carcinomata) sub sequently developed in twelve mice which were not more than three months old Typical lymphosarcomata originating in the lymph nodes were also observed, and in seven cases the leukæmia characteristic of the strain occurred but with the difference that all the animals dying from this cause were less than five

Furth, Furth and Breedis (1938) examined three of these strains (S1b, Rf, and A, with sub lines Af and Ak) The mice were treated with 0 05 c cm of a 4 per cent solution of benzpyrene in lard, injected into the spleen Closely related animals were used in the investigation, one half being given the treatment and the others kept as controls Only a small number—seven and eight respectively—from strains S1b and Ak, was examined, but forty eight animals from strain Rf were treated Twenty three cases of tumour of the lungs were found among these, as against nine among forty nine control animals, ten cases of leukæmia also occurred, against one in the same number of control animals Of the ten leukæmic cases, seven were monocytic and two myelogenous A number of tumours developed in twenty two animals of strain Af—three tumours of the lungs and three mammary tumours—but only one leukæmic case (monocytic leukæmia) occurred One case of lymphogenous leukæmia was seen in twenty nine control animals The special (intrasplenic) method of administration seems to have been responsible for the positive result, and in particular for the occurrence of the rare monocytic form of leukæmia, which was especially evident in strain Rf Such cases also occur spontaneously in this strain, though far less frequently On the other hand, the treatment does not seem to have increased the incidence of leukæmia in strain Af

The cases of monocytic leukæmia observed were characterised by an overgrowth of fairly large and somewhat irregular mononuclear cells, especially in the liver, and to a lesser extent in the spleen and lymph nodes The cells now and then formed small nodules in the liver and spleen No blood changes were found Varying numbers of large cells even polynuclear giant cells, were observed between the cells in the affected

organs, the picture thus to some extent resembling that of Hodgkin's disease The leukæmic nature of the disease may perhaps be doubted, even the authors calling it 'a leukemia like disturbance with malignant cells resembling monocytes' As already mentioned, similar pictures are seen, though rarely, in untreated animals of strain Rf and, according to the authors, the effect of the treatment was mainly evidenced by the fact that 'the incidence of this disease was greatly enhanced by the treatment with benzpyrene' The experiments certainly showed a distinct increase in the number of cases of this condition in the animals treated, and the fact that the disease was of a type that is rare in spontaneous cases may have been due to the method of administering the benzpyrene

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months old Both leukæmic and aleukæmic cases were observed

These experiments were continued by Engelbreth Holm and Lefevre (1941) and it has appeared that the method of administration is of only minor importance, for painting the skin of the back with a 0 5 per cent solution of 9 10 dimethyl 1 2 benzanthracene in benzene, two or three times a week, led to similar results Leukæmic or lymphosarcomatous changes, occurring at an earlier age than in untreated animals, were observed in ten out of eleven mice of this strain which had survived the first months of treatment This was in addition to tumours—due to the painting—of the usual papillomatous or carcinomatous type A specially interesting point in this experiment was that lymphatic infiltrations developed at the site of painting in five of the animals They were highly reminiscent of lymphosarcomata, with pronounced infil trative extension into the underlying tissue (see Fig 44) The authors concluded from these experiments that leukæmia of spontaneous development can be accel erated so as to occur at an earlier age than usual, by treatment with carcinogenic hydrocarbons, and that the lymphatic tissue of animals belonging to this strain has a greater tendency than that in other mice to react to carcinogenic agents by developing malignancy This is evident from the lymphosarcomatous infiltration *at the site of painting, even in three mice in which true* leukæmia was not observed

Engelbreth Holm and Lefevre also examined mice of the Little dilute brown strain, with results partially similar to those of Morton and Mider (p 179) Experi ments resembling those mentioned above were performed with this strain, in which 50 per cent of the animals develop mammary carcinoma at the age of eight to ten months without treatment and 2 per cent leukæmia



FIG. 44. Squamous cell carcinoma in skin of mouse of strain Ak. after painting with 9:10-dimethyl-1:2-benzanthracene. Considerable infiltration of a lymphosarcomatous nature is seen in the stroma. × about 50.

when they are over six months old The results were as follows Of nineteen animals treated with 9 10 dimethyl 1 2 benzanthracene (½ mg), four succumbed to lymphogenous leukæmia and one to lymphosarcoma All were less than six months old when they died In addition, the development of mammary cancer was observed in three animals at considerably earlier ages than in the controls Among thirty three animals painted in the ordinary way, seven cases of leukæmia developed at ages less than six months, and seven cases of mammary carcinoma (three of which were multiple) below the age of eight months As in Morton and Mider's experiment in which methyl cholanthrene was used, distinct acceleration was observed of the leukæmia which occurs so rarely in this strain evidenced both by a distinctly increased incidence (eleven out of fifty two animals against 2 per cent of those not treated) and by earlier occurrence Moreover, there was a corresponding acceleration of the mammary adenocarcinomata typical of the strain, of which up to five or seven per animal have been found in other series of experiments in which methyl cholanthrene was used

Thus treatment with carcinogenic hydrocarbons, injected subcutaneously or painted on the skin, produced in both the Aka and 'dilute brown strains an acceleration of the forms of tumour usually encountered therein The 'accelerated' leukæmias in each of these strains proved transmissible to animals of the same strain just like the spontaneous cases in untreated animals

Mice of Bagg s strain were used by the same authors in similar experiments Only one case of lymphogenous leukæmia developed among twenty seven animals given 9 10 dimethyl 1 2 benzanthracene subcutaneously This result was therefore, in striking contrast to Brues and Marble's experiments (p 180), in which

50 per cent of the mice of the same strain developed leukæmia after being painted with tar

Further confirmation of the 'accelerating effect' of carcinogenic hydrocarbons has been obtained by Kirschbaum, Strong and Gardner (1940) However, MacDowell *et al* (1938) found that benzpyrene had no accelerating effect upon the incidence of leukæmia in their strain C 58 It must be remembered that this strain is the most susceptible known up to date, and it may be that any further enhancement is impossible

EXPERIMENTS WITH X RAY IRRADIATION

Leukæmia can be produced experimentally in mice, or accelerated (the latter term is preferable), by means of irradiation with X rays, as well as by the influence of tar and carcinogenic hydrocarbons

Krebs, Rask Nielsen and Wagner (1930) observed only three cases of leukæmia (lymphogenous) in 10,500 non treated, non inbred mice of mixed origin, whereas nineteen cases of lymphosarcomatous change or lymphogenous leukæmia were seen in 5550 animals that had been exposed to total irradiation with X rays

Furth and Furth (1936) similarly irradiated a large number of mice of strains A, R and S with from 200 to 400 r when they were 5 10 weeks old The incidence of the neoplastic diseases that were seen also in the untreated animals was thereby increased It is particularly interesting that myelogenous leukæmia occurred in the irradiated animals from five to nine times as frequently as in the untreated Lymphogenous mediastinal tumours occurred three times as frequently in the irradiated animals in strain R as in the non irradiated, and in strains A and S eight times as frequently The cases observed in the irradiated animals were in no way different from those seen in the others General lymphogenous leukæmia was twice as frequent in the

irradiated as in the non irradiated animals of all three strains

A considerable increase in the incidence of leukæmia can thus be attained, both in stocks of mixed mice and in the purer strains, by means of irradiation with X rays. This increase is comparable with that observed after treatment with carcinogenic substances

EXPERIMENTS WITH ŒSTROGENIC SUBSTANCES

As with mammary carcinomata, it seems that the frequency of leukæmic diseases can be increased, or the diseases themselves produced in certain strains by means of œstrogenic substances. Lacassagne (1937) reported fourteen cases of lymphosarcoma in mice which had been treated with weekly injections of equilenin benzoate, in doses corresponding to about 500 M U per injection. He stated that spontaneous occurrence of these tumours had not been observed for five years in the strains of mice employed

DISCUSSION OF RESULTS

It has been mentioned already (pp 115 and 151) that the development of leukæmia has been accelerated in mice by the injection of embryonic extracts (MacDowell, Potter and Taylor, 1937), and by the injection of assumedly cell free extracts of leukæmic tissue (Engelbreth Holm and Frederiksen, 1938*b*). But it is impossible as yet to form any definite opinion as to whether the accelerated development of the morbid processes attained in these experiments is similar to, or different in principle from, that which can be attained by treatment with œstrogenic substances, carcinogenic hydrocarbons and X rays

That leukæmia can be caused in mice by means of tumour producing influences is beyond discussion. The question then remains —How are we to regard the

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Furth and Furth (1936) similarly irradiated a large number of mice of strains A, R and S with from 200 to 400 r when they were 5 10 weeks old The incidence of the neoplastic diseases that were seen also in the untreated animals was thereby increased It is particularly interesting that myelogenous leukæmia occurred in the irradiated animals from five to nine times as frequently as in the untreated Lymphogenous mediastinal tumours occurred three times as frequently in the irradiated animals in strain R as in the non irradiated, and in strains A and S eight times as frequently The cases observed in the irradiated animals were in no way different from those seen in the others General lymphogenous leukæmia was twice as frequent in the

is not necessary for the occurrence of any leukæmia at all, even with the strongest extrinsic influence possible This cannot yet be decided with certainty The experiments of Brues and Marble may, however, point in that direction, since two inbred strains reacted very differently to the same influence, one developing a 50 per cent incidence of leukæmia, the other not a single case Neither of the strains was a 'leukæmic strain' The interpretation of this observation may conceivably be that there are strains in which leukæmia cannot be produced at all, a question the closer investigation of which will be of great importance In this connection it is interesting to note that, in most of the strains in which the experimental production of leukæmia has succeeded cases of leukæmia have also been observed among the untreated animals, though extremely rarely in some of them

Lewis (1938) stated expressly that no spontaneous leukæmia is known in strain C3H in which 1 2 5 6 dibenzanthracene produced three lymphosarcomata among over a hundred animals Dobrovolskaia Zavadskaia and Rouyer (1938) reported the same about their lignee XXX in which three cases of lymphadenosis were produced among 153 animals but these authors gave reasons for believing that a hereditary disposition co operated in the occurrence of such cases

The view that a certain hereditary predisposition is a condition for the experimental production of leukæmia is perhaps supported by the fact that the cases produced in the experiments are preponderantly of the same type as those which also occur spontaneously in the strains concerned the various types of leukæmia being inherited individually in certain inbred strains, such as those used in Furth's experiments Among these lymphogenous leukæmia is by far the most frequent form in one strain (Ak) and myelogenous in another

relation which seems to exist between the spontaneous hereditary tendency to develop the disease and the tendency to react to the external tumour producing influences with increased or accelerated occurrence of the same morbid processes? The further question arises Can the conclusions drawn from the leukæmia investigations already quoted be considered valid also for other kinds of tumours?

In the discussion of MacDowell and Richter's investigations into the heredity of leukæmia in inbred strains of mice (Chapter VI), it is emphasised that an interplay must be assumed between the intrinsic, genetic factors and the extrinsic factors which contribute to the development of leukæmia This point is illustrated by a strain in which the animals were genetically uniform as regards the development of leukæmia, but in which 10 per cent failed to develop the disease These leukæmia free animals had—as was evidenced by their offspring—the same genetic constitution in respect of leukæmia as the other 90 per cent, so that the failure to develop the disease must presumably have been due to extrinsic influences, or to the lack of them Analogous reasoning in the case of another strain, only 2 per cent of the animals of which developed leukæmia, led to the assumption that the development of the disease in these must have been due to extrinsic influences

The observation of an accelerated or a numerically augmented tendency to leukæmia in these strains under influences of a carcinogenic nature is quite compatible with such assumptions *Whereas the intrinsic, hereditary* characteristics of the untreated animals ordinarily play by far the larger part, the extrinsic factors can be increased experimentally to such a degree that the hereditary characteristics play a relatively small part in the *leukæmias produced in such experiments* But it is a question whether a certain hereditary constitution

or the myeloid system in consequence of inherited characteristics, as we do of those produced by treatment with carcinogenic or œstrogenic substances, or X rays, and we are still without knowledge as to whether these intrinsic and extrinsic influences on the cells are similar in nature or essentially different, though they co operate in the production of malignancy

We know that various factors which can cause the development of tumours individually can intensify each other s action when they are applied simultaneously this has been shown by Mottram (1938) with radium irradiation and painting with benzpyrene, by Rous and Kidd (1936 1938) with tar and the Shope papilloma virus by Andrewes and Ahlstrom (1938) with tar and the Shope fibroma virus by Clemmesen (1938) with X ray irradiation and the Shope fibroma virus * by Mayneord and Parsons (1937) with X ray irradiation and treatment with carcinogenic hydrocarbons and finally by Perry and Ginzton (1937) with dibenzanthracene and theelin But we do not know whether the co operation between the inherited tendency towards leukæmia and the extrinsic carcinogenic influences is of an analogous nature or whether the hereditary tendency is not rather a factor of quite another kind whose presence is possibly an absolute condition for the successful action of the extrinsic tumour producing influences

We have learnt that carcinogenic hydrocarbons X rays and probably, œstrogenic substances are some of the extrinsic non hereditary factors that may be of importance—in some cases even of overwhelming importance—in the development of leukæmia It is

* Clemmesen emphasized that it is possible and in some cases perhaps even probable that irradiation in combined experiments such as these should be regarded not as a carcinogenic influence *per se* but only as a factor lowering resistance the action of which is analogous to that seen when resistance to transplanted tumours is broken down by X ray irradiation before inoculation

(Rf), whereas both types are observed in a third (S) (Barnes and Sisman, 1939)

The features of leukæmia are presumably analogous to those of other forms of tumour For instance, a hereditary disposition has been shown many times to be important in the development of tumours produced by painting the skin The production of mammary carcinoma by means of œstrogenic hormones may be mentioned as a striking analogy Lacassagne (1938) showed that treatment with œstrogenic substances will accelerate the appearance of mammary carcinoma in inbred strains in which these tumours are observed spontaneously, but not in other strains which are free from mammary cancer The acceleration manifests itself in an increased number of tumours and also in their earlier development This acceleration is thus quite similar to that attained by means of carcinogenic hydrocarbons in leukæmic strains An increased number of tumours, and their earlier development, are observed in both instances, but are confined in each case to strains in which the same type of tumour is seen spontaneously The very different reactions of various kinds of animals to the same influence may possibly be regarded as an indication of genetically controlled constitutional differences in susceptibility to the neoplastic diseases For example, the attempted production of carcinomata or even of papillomata, by painting with tar was unsuccessful in the case of fowls, and, again, many differences of reaction are observed even among the various kinds of rodents Similarly, it may be that the production of leukæmia, such as is seen in mice, will never be attained in rabbits, for instance, in which spontaneous leukæmia in its typical form is not known

We know just as little of the changes in the direction of malignancy which occur in the cells of the lymphatic

or the myeloid system in consequence of inherited characteristics, as we do of those produced by treatment with carcinogenic or œstrogenic substances, or X rays, and we are still without knowledge as to whether these intrinsic and extrinsic influences on the cells are similar in nature or essentially different, though they co operate in the production of malignancy

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probable that other influences may play a part, but we do not know what they are Young (1922) and Cherry (1929) thought they had observed that such an influence was exerted by bacteria (among others by the tubercle bacillus), their experiments, however, did not uphold their contention, as was proved by Richter and MacDowell (1935) Similarly, Dobrovolskaia Zavadskaia and Rouyer (1938) believed they had observed the development of leukæmia after infection with *Spirochæta morsus muris* Such claims, however, must be regarded with scepticism, so long as a purely hereditary origin is not entirely out of the question and perhaps especially so long as it has not been proved convincingly that the changes in question are leukæmic and not merely leukæmoid reactions, such as are of common occurrence in infections in mice

PART V

CHAPTER X

NATURE OF THE ANIMAL LEUKÆMIAS

OPINIONS concerning the nature of the morbid processes in the animal leukæmias have, as is only natural, passed through the same stages as those concerning human leukæmias Research on the former has however, resulted in the now widely accepted opinion that these diseases both in animals and in man, are to be regarded as neoplastic

The *infection* theory was abandoned after a comparatively short time Even though Ellermann and Bang again raised this hypothesis for fowl leukæmia, after their discovery of a virus that transmits the disease, it was not long before it became clear that leukæmia cannot justifiably be regarded as an infectious disease either in fowls or in mammals Almost all the features typical of infectious diseases are lacking in leukæmia The disease is not contagious and the anatomical changes in the organs present no characteristics that can justify their being considered as inflammatory

These facts are not altered by the extremely rapid progress of some of the cases in which the pathological picture may be reminiscent of sepsis still less are they affected by the occasional demonstration of microbes in a leukæmic lesion, which is most often due to secondary septic infection in an organism weakened by leukæmia The not uncommon leukæmoid reactions which as mentioned before occur in various infections—especially in fowls and mice—have also helped to confuse attempts to define the nature of the leukæmic process, as they

have not infrequently been erroneously diagnosed as leukæmia In some cases the microbes concerned (generally streptococci) have even been acclaimed as the cause of leukæmia, because of the somewhat similar pathological pictures they produce when inoculated into other animals, generally mice These pictures, however, have nothing to do with leukæmia, they too are leukæmoid reactions to septic infections

Nor has Ziegler's *correlation* theory proved tenable This hypothesis, which was purely speculative, was based on the normal existence of a balance between the lymphogenous and myelogenous systems, and the suggestion that if one of these systems was for any reason damaged or had its development inhibited, hyperplasia of the other would occur Repression of the lymphogenous tissue is certainly often observed in myelogenous leukæmia, and *vice versa*, but it by no means follows that this disappearance of the elements of one system is primary, still less that it is the cause of the leukæmic proliferation of the other This hypothesis must also be regarded as abandoned

The *tumour* theory first advanced by Ribbert in his *Geschwulstlehre* (1904), is based on the assumption that leukæmia is a tumour formation of the myelogenous or lymphogenous tissue Since its first publication this hypothesis has sometimes been discredited Investigations of the animal leukæmias during the last ten years however have made it clear that leukæmic changes are neoplastic in type, although the question whether the growth is a true malignant tumour, or whether leukæmic proliferations should be likened rather to benign hyperplasias is still open to discussion Whether or not lymphogenous leukæmia be regarded as a benign hyperplasia, the corresponding undoubtedly malignant tumour is certainly the lymphosarcoma Now it appears unmistakably, from the experiments with mouse

leukæmia which have been described above, that no clear cut border line can be drawn between lymphogenous leukæmia and lymphosarcoma in mice, and that the former, at any rate in these animals, must, like the latter, be recognised as a malignant new formation

The diffuse nature of leukæmic changes, in contrast to the more defined, nodular formation in 'true' tumours, has been especially urged as an argument against the tumour theory That there is a difference between the leukæmias and other malignant tumours is incontestable, but it is extremely doubtful whether one is therefore justified in characterising leukæmia as fundamentally different from other tumours Many cases of leukæmia have been known in which the changes were not general but were localised to more or less limited regions of the system concerned, and cases characterised by quite localised nodular formations (lymphosarcoma, myeloma, etc), of whose neoplastic nature there can hardly be any doubt, are also not unusual Again a comparison between leukæmia and tumours in other tissues cannot be made unless the normal differences between the blood forming tissues and blood cells on the one hand and the non blood forming tissues on the other are taken into consideration The blood forming tissues occupy a special position among the tissues of the organism in being scattered, with no sharp boundaries, and especially in producing cells that lack the ability to form cohesive tissue If a hypothetical malignant new formation be imagined as proceeding from these tissues the result can hardly be thought to be other than the very picture that is seen in typical generalised leukæmia

Another point which has been emphasised by the opponents of the tumour theory is that leukæmic tissues do not metastasise It is true that a typical

primary tumour and scattered nodal metastases are not found in leukæmia, but it is extremely doubtful whether this means that the changes are not of a neoplastic nature, for the characteristic structure of the hæmopoietic tissues, made up of isolated cells, will cause the supposed primary focus and the 'metastases' proceeding from it to spread and not to appear as compact nodules. Moreover, this question of whether metastasis does or does not occur in leukæmic processes is still open to debate. It has not yet been definitely decided whether the changes which spread universally throughout the organism in both myelogenous and lymphogenous leukæmia are to be considered as indicating that the whole system, once and for all, undergoes conversion to malignant tissue, or whether it should be assumed that leukæmic processes arise in one place, perhaps even in one cell in the system concerned, and then spread throughout the organism as a diffuse metastasis of isolated cells. Arguments have been advanced in support of both theories.

As already mentioned, it appears from experiments on leukæmia transplantation into mice that diffuse leukæmic processes can be initiated by the intravenous *inoculation of a single cell. Cases of leukæmia are not* infrequently seen in mice—as also in man—in which only single sections of the lymphogenous system become leukæmic. Whether these are to be regarded as cases in which general changes would have appeared, had the individual concerned lived longer, can hardly be decided, but they show, at any rate, that in cases of leukæmia all the sections of a hæmopoietic system need not be leukæmic simultaneously.

Although these observations indicate that the changes begin relatively locally, later to become 'generalised', they do not necessarily mean that the leukæmic process arises by the metastasis or colonisation of leukæmic

cells from a primary leukæmic 'tumour' In this connection several facts indicate that in leukæmia the hæmopoietic tissues undergo certain changes throughout the whole of their extent, which may mean that the disease itself is systematised from the very beginning, and here the metabolic changes found in Potter and Victor's experiments in the lymph nodes of mice, at periods when there was no other evidence of leukæmia, come to mind Moreover, experiments in which the development of spontaneous leukæmia in mice has been accelerated by local treatment with carcinogenic hydro carbons indicate the presence of such a general preleukæmic state since the manifest leukæmic changes in such cases have been found earliest and most pronounced on the sites selected at random where the carcinogenic influence has been active As already mentioned by painting the skin lymphosarcomatous infiltrations can be caused to develop subcutaneously in animals that would later have developed lymphogenous leukæmia spontaneously

The truth lies, perhaps midway between these two opinions It would not be unreasonable to suppose that the inherited tendency which has been shown to be of the greatest importance for the development of leukæmia in mice determined a change or state, pre leukæmic in nature throughout the whole of the lymphogenous or myelogenous system As a result of exogenous influence or influences it might then be thought that the real leukæmic process arose in a limited area in the hæmopoietic system The further development of this process—generalisation—might be supposed to take place just as much by metastasis as by the transformation, under the same exogenous influences of other sections of the system in a leukæmic direction Such a multicentric origin of neoplastic processes is known in the case of other tissues, although

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it is rarer than isolated tumours As examples, mention may be made of Krompecher's carcinomata in the skin, which are frequently multiple, bilateral suprarenal and ovarian carcinomata, multiple intestinal carcinomata neurofibromatosis etc

It may be argued, then, from the foregoing that the peculiarities which distinguish leukæmia from other tumours, and are considered by the opponents of the tumour theory to disprove the neoplastic nature of leukæmia, can be easily explained as consequences of the special features which normally characterise the hæmopoietic tissues Moreover, recent research on leukæmia in animals has supplied a large number of observations which directly support the theory that leukæmic processes are malignant tumour formations

According to the extensive investigations of recent years (Furth and co workers Jarmai, Oberling and Guerin, Engelbreth Holm, Rothe Meyer and co workers, and others) there is in the fowl leukæmias (erythro leukæmia, myelogenous leukæmia and lymphogenous leukæmia) an unrestricted multiplication of blood cells in their earlier stages, accompanied by a lack of ability to mature Where these cells appear in the tissues they form infiltrations and localised nodes here and there (erythroblastomata, myelocytomata lymphosarcomata) which possess all the characteristics laid down for malignant tumours The individual cells in the infil trations and in the blood similarly present morphological and other features supporting the view that they are tumour cells As in the case of many other malignant tumours pronounced enlargement of the cell nuclei, or rather an alteration in the ratio nucleus / cytoplasm (and especially of the ratio nucleolus / nucleus), is often found For instance, the nuclei of the basophil erythro blasts of fowls with erythroleukæmia are undoubtedly larger than the nuclei of the normal basophil erythro

blasts (Engelbreth Holm, 1933), and the nucleoli in the pathological cells are also surprisingly large

Such features are quite compatible with the tumour theory, though they hardly prove it Nor have attempts to produce fowl leukæmia experimentally with tar or carcinogenic hydrocarbons given such uniform results that the neoplastic nature of the disease has been thereby established, although many of the cases apparently so 'produced' have probably been authentic

But the study of the mixed leukæmia sarcoma strains has shown, with all the clarity desirable, that fowl leukæmia and fowl sarcomata are closely similar diseases since they appear in many strains alternately or simultaneously and are produced by the same virus The results of experiments with such strains as those of Furth, Oberling and Guerin, and Rothe Meyer and Engelbreth Holm leave no room for doubt as to the neoplastic nature of fowl leukæmia, unless it be doubted whether the virus produced fowl sarcomata are themselves malignant tumours Thus no differences have been demonstrated between the properties of the viruses producing pure sarcomata *e g* the Rous tumour no I, and those of the viruses causing the development of pure leukæmia, or leukæmia combined with endothelial sarcomata or other tumours There exist indeed many fowl tumours caused by viruses, including fibrosarcomata myxosarcomata, endotheliomata and the leukæmias with forms of transition from one to the other

In the same way, the sum total of observations on the leukæmias of mammals, especially the mouse leukæmias necessitates the assumption that neoplastic processes are involved

As with the bird leukæmias, the typical changes in the tissues observed in mammalian leukæmias present features strongly indicating that the cells are neoplastic There is no question here of normal immature blood cells,

but rather of atypical cells with special morphological characteristics which are preserved consistently throughout transplantation passages The invasive growth of the infiltrations into the surrounding tissues and the characteristic features of the individual cells, especially their lack of ability to differentiate, which has been established by the studies of MacDowell, Victor and Potter, and of Furth and his co workers, and which is typical of neoplastic tissue, can hardly be interpreted on any other supposition than that the cells are tumour cells with malignant characteristics

Cells from the leukæmic tissue of mice further display certain metabolic anomalies which are essentially of the same sort as those found with other tumour cells The facts in this connection are, however, somewhat difficult to evaluate, because from a metabolic point of view the blood cells deviate even normally from most of the other cells of the body

The problem of the nature of the morbid process in mammalian leukæmias can hardly be solved by investigations of cell metabolism, and by morphological studies alone As in the fowl leukæmias, we meet with difficulties arising from the peculiarities of the tissues concerned, and certain features in the leukæmic processes give them a position among the tumours which is in some respects unique But, just as the problem of fowl leukæmias was solved indirectly through the results attained from mixed strains, so has the nature of the tumour in mouse leukæmias become apparent from experiments in which leukæmia was produced experimentally

Leukæmia is inherited in inbred strains of mice in which the frequency of various forms of the disease has become constant through a series of generations, in the same way as tumours By means of inbreeding, 'leukæmia strains' are produced in which one form

or another of the disease predominates, while strains that are free from leukæmia are also produced It has been shown that an interplay of intrinsic (inherited) and extrinsic factors is necessary for the occurrence of leukæmia, as for that of other malignant tumours Transplantation experiments with strains (such as those of MacDowell and his co workers, and of Furth and his co workers) have also shown that the same facts apply to leukæmia as to other tumours, as regards the conditions for transmission and the development of immunity to the transplanted tissue On no essential point have differences been demonstrated between the leukæmic strains and other strains of tumours in mice, so that it would be unreasonable to consider that mouse leukæmia is not of a neoplastic nature The final and incontestable proof of this contention is supplied by the experiments mentioned at p 183 in which leukæmia was produced experimentally by means of carcinogenic agents While the epithelial tissue reacted with the development of carcinoma, and the connective tissue with sarcoma, the hæmopoietic tissue reacted with leukæmia

The carcinogenic hydrocarbons have proved, in the case of leukæmia as with other malignant tumours, to be the most effective of all the known exogenous factors which may combine with hereditary predisposition to produce malignant transformation of the cells in a tissue They are able to accelerate the manifestation and to increase the number of cases of the disease in inbred strains in which the incidence of leukæmia is already relatively high

Other influences capable of co operating in the development of malignant tumours can also cause an increase in the number of cases of leukæmia in these strains This has been proved for X ray irradiation and, in a single experiment, for œstrogenic hormones

The most important result of recent leukæmia research has thus been that both animal and human leukæmias have been almost unanimously recognised as neoplastic diseases, as malignant tumours of the hæmopoietic tissues * Leukæmia research has thereby become a link in general tumour research, and the results which may be attained in future from the investigation of leukæmic diseases will presumably be of value in relation to the understanding of cancer and other tumours

THE RELATION BETWEEN HUMAN AND ANIMAL LEUKÆMIAS

The relation between human and animal leukæmias has been a subject of discussion and argument since the observation of the first case of animal leukæmia Warnings have often been given against comparing human diseases with those of the lower animals 'Mice

* On the basis of tissue cultures of bone marrow taken from human cases of acute myelogenous leukæmia Israels (1940) suggests that there may be a fundamental difference between leukæmia in mice and in man In the former case he admits that the disease is neoplastic in the latter he disputes this view on the grounds that immature cells rapidly disappeared from his cultures which soon consisted almost entirely of more mature forms There are several reasons for rejecting Israels's argument Very similar results are obtained with tissue cultures of the admittedly neoplastic mouse leukæmias The malignant cells die out rapidly and explants soon contain nothing but fibroblasts and mature myeloid cells (MacDowell *et al* 1938) Even if it be true as Israels believes that the observed result is due to differentiation of myeloblasts this does not disprove the neoplastic nature of human leukæmia A squamous celled carcinoma differentiates and Potter maintains that the malignant cells of mouse leukæmia al o do so (p 152) The argument would be conclusive only if mouse and human leukæmias were studied in tissue culture side by side and were shown to be fundamentally different It is possible however that certain cases of leukæmia though clinically typical may not be fully neoplastic MacDowell *et al* (1939) have described the induction of such border line cases in mice following the injection of sterile trypan blue Such conditions would correspond to the carcinomatoids etc and serve only to accentuate the general similarity between leukæmia and other neoplastic diseases

are not men' is a familiar truism, and on these grounds the experimental study of animal leukæmia has been described by its opponents as purely 'academic' and of no practical significance to human medicine Such views can hardly be justified It is obvious that there are differences between fowl leukæmia, for instance, and human leukæmia, just as there are characteristic differences between the hæmopoiesis of fowls and that of man, but it has been established almost beyond peradventure that fowl leukæmia and human leukæmia are analogous diseases Nearly all who have engaged in research on these subjects in recent years are agreed on this point But the analogy between mouse leukæmia and human leukæmia is still more striking The similarity here is remarkable—so perfect, right down to the smallest detail that doubt as to the identity of the two diseases can hardly be entertained The clinical course in acute human cases and that seen in those with a slower rate of progress, are repeated in mice, and the pathological picture is amazingly uniform in both The same forms of leukæmia are found—lymphogenous and myelogenous most frequently monocytic leukæmia and the atypical forms, *e g* cases characterised by megakaryocytic elements, more rarely—in mice as in man

Only one form of human leukæmia — plasma cell leukæmia—and the related multiple myelomatosis, have not yet been observed in lower animals Whether this is a pure matter of chance—*i e* whether these conditions do occur in animals but have not yet been observed—or whether myelomatosis and plasma cell leukæmia are peculiarly human forms cannot yet be decided the latter explanation would hardly appear likely

Altogether there can be no legitimate grounds for doubt that leukæmia in animals especially in mice, is the same disease as leukæmia in man The peculiar

features presented by the disease in some species, especially birds, are more reasonably explained as being due to the special characteristics of these species than to the possibility that fowl leukæmia, for example, is a disease of an entirely different nature from leukæmia in other animals or in man

With the solution of the problem of human leukæmia as the final goal, the study of the nature of the disease and of its various features in animals must therefore be regarded as abundantly justified, since the results attained are likely at the same time to elucidate the corresponding features of leukæmia in man

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